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THE IMPACT OF ULTRAVIOLET WAVELENGTHS ON BROILER CHICKEN PERFORMANCE, HEALTH AND WELFARE

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IV. ABSTRACT

Qualities of the light environment affect the performance, health and welfare of broiler chickens. UVA light is visible to chickens and may facilitate improvements in welfare. UVB wavelengths promote endogenous vitamin D synthesis, which could support the rapid development of broilers. The aim of the study was to investigate the impacts of Ultraviolet wavelengths (UV) on performance health and welfare indicators.

Day-old Ross 308 birds (n = 638) were randomly assigned to one of three lighting treatments: A) White Light Emitting Diode (LED) & supplementary UVA LED lighting (18-hour photoperiod); B) White LED with supplementary UVA & UVB fluorescent lighting providing 30 micro watts/cm^2 UVB at bird level (for 8 hours of the total photoperiod to avoid over-exposure of UVB); C) White LED control group, representative of farm conditions (18-hour photoperiod). Birds were fed a commercial diet and kept at a final stocking density of 33kg/m^2.

Indictors measured were: (Performance) average daily gains, mortality, final weights, breast weights and leg weights. (health) bone mineral density, leg composition, bone measurements, tibia strength and severity of tibial dyschondroplasia. (welfare) feather condition, tonic immobility duration and walking ability, using the Bristol Gait Score.

Growth was faster in male broiler chickens in treatment B, though slower in males in treatment A. Similar final weights were achieved in all treatments. Treatment A and B improved gait score, additionally heavier broilers in both treatments had improved walking ability compared to control broilers of similar weights. Treatment A also reduced fearfulness. There was no impact of either treatment on skeletal or ocular health measures. Together these results suggest UV wavelength supplementation may offer a promising husbandry refinement for commercial indoor lighting regimes; offering potential benefits to both bird welfare without compromising performance.

V. GENERAL INTRODUCTION

V. I) THE DEMAND FOR ANIMAL DERIVED PROTEIN

Global Food Security is said to exist when "all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life". (World Food Summit, 1996). Achieving global food security in the face of climate change is one of the major challenges faced by both developed and developing nations (Schmidhuber & Tubiello, 2007; Godfray, *et al.,* 2010 ; FAO, 2016). Meat products represent a high-quality source of protein and the demand for affordable meat is increasing (figure V.I). The Food and Agriculture Organization (F.A.O) predicted global consumption would rise by 102 % from 2006 to 2050 (F.A.O, 2006). This translates to an additional demand of 233MT of meat. In developing countries there has been a demand driven "livestock revolution", with meat consumption increasing by three times that of developed countries between the 1970s to the mid 1990s due to urbanisation and economic growth (Delgado, *et al.*, 2001; Delgado, 2003).

Current animal production systems have been criticised as major contributors to climate change and a threat to biodiversity (Gerber, *et al*., 2013), though the production of chicken meat has a smaller environmental impact compared to other sources of animal meat (Vries & Boer, 2010; Herrero, *et al*., 2013). Reducing or even eliminating meat from diets is often suggested as necessary to reduce the environmental impacts of animal production, though consumers may be resistant to these suggestions (Graça, *et al*., 2015; Macdiarmid, *et al*., 2016). Chicken meat is not associated with social or religious taboos and is generally viewed as a cheaper, healthier option than red meats such as lamb and beef (Bentley & Buzby, 2012; Priceonomics, 2013).
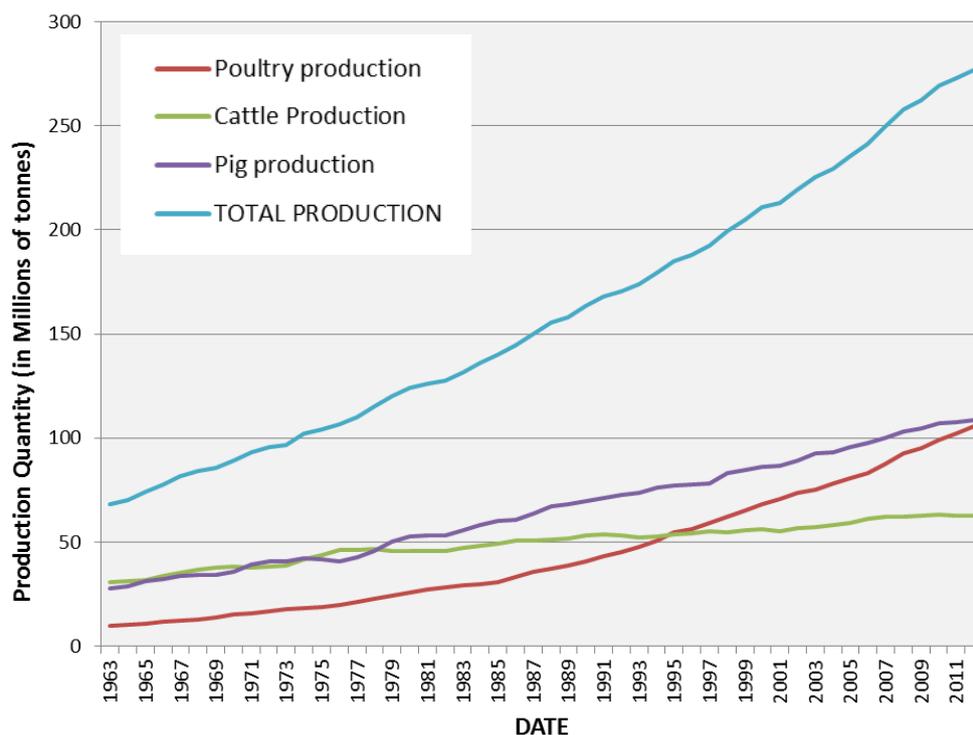
Figure V.I – Global meat production

The increasing global production of meat in millions of tonnes for poultry (including chickens, turkeys, ducks and geese), cattle and pigs together with the total. In 1963 poultry meat represented 14% of the total meat production quantity compared to 38% in 2012. It should also be noted that chicken meat represents an average of 87% (± 1.04) of the poultry production quantity across the time scale shown. All data are from FAOSTAT 2014.

The demand for poultry meat has increased consistently at a rate of three times that of population growth (FAO, 2013), and the UK population alone consumed 31.2kg per capita in 2014; more than any other meat consumed (BPEX, 2014). Based on this evidence, chicken meat is likely to represent a large proportion of the future demand for meat and the broiler chicken will be the focus of the work presented in this thesis.

V. II) THE STRUCTURE OF THE MODERN POULTRY INDUSTRY AND THE CHALLENGES FOR BROILER CHICKEN WELFARE

The red jungle fowl, modern forms of which are found today in central and south India, Myanmar, Malaysia, Thailand and Cambodia, is the species of origin of many modern breeds and strains of poultry (Appleby, *et al.*, 1992). Domestication of this tropical species occurred around 2500 BC when birds were predominantly reared for religious, cultural and entertainment purposes and not as a food source (Rose, 1997). In the 1850s there was a great interest in the domestication of poultry and many specimens with distinct breed standards emerged, but the focus was primarily on breeding show birds and not on enhancing productive performance (Appleby, *et al.*, 1992). The poultry industry began to expand in the 1900s and divided into specialized and distinct sectors including egg production, meat production and those involved with the rearing and breeding of commercial stock.

The primary breeding sector, which supplies the production sector, has become progressively dominated by a small number of large multinational companies which distribute Pedigree and Grandparent stock across the world. For example, 90% of the UK broiler stocks are supplied by two of the largest international breeding companies (DEFRA, 2006). The parent stocks are purchased by companies or hatcheries that supply the production sector. In 1990 most broilers in the UK were in the hands of 12 integrated companies supplying the poultry meat market (Appleby, *et al.*, 1992). In comparison, by 2004, 70% of UK broilers were processed by just 4 companies, who themselves produced half of all broilers on their own holdings (Sheppard, 2004). The average flock size in developed countries has also increased, with 97 % of UK broiler chickens housed in flocks of more than 20,000 birds in 2002 (Sheppard, 2004).

The broiler chicken itself was based on a hybrid of the Plymouth and Cornish white rock breeds, which were developed in the USA for their rapid growth

rates and efficient feed conversion ratios. Chicks weighing just 45g at hatch can reach finishing weights of approximately 2.2kg in 42-45 days allowing for 6 or 7 crops of broilers to be produced in a year.

The rearing of broiler chickens in the UK is governed by many regulations that aim to protect the animal's health and welfare. These regulations cover: the training of animal husbandry staff, provision of food and water, routine inspections, veterinary care, farm biosecurity, limits on stocking densities and environmental requirements such as maintaining acceptable temperatures, litter quality, lighting conditions, and ventilation. (DEFRA, 2011).

The biggest expense of managing poultry is the cost of feed, which accounts for around 60% of total costs (Sheppard, 2004). For this reason, the improvement of feed conversion ratios in broilers has been a key area of research to maximise economic performance alongside selective breeding for rapid growth rates. However, these breeding polices, in combination with intensive farming practices, have been accompanied by significant health and welfare problems including: increased incidences of musculoskeletal disorders and lameness, contact dermatitis, metabolic disorders such as sudden death syndrome, and high rates of infectious diseases due to the challenge of disease control and treatment in intensive farming systems (Julian, 1998; European Commission, 2000; Weeks & Butterworth, 2004; Bessei, 2006).

The productivity and economic performance of broiler chickens are important but should not be considered in isolation. Increasingly there is scientific and public interest in improving the sustainability of production while safeguarding the health and welfare of broiler chickens.

In 1946 the World Health Organisation (WHO) defined health as a "state of complete physical, mental and social well-being and not merely the absence of disease of infirmity" (WHO, 2020). Thus, mental health and emotional well-being have long been considered key aspects of overall health and likewise physical health is also linked to emotional well-being. However, In veterinary and animal science fields, aspects of 'mental health' or attempts to understand

and measure the emotional experiences of animals have only been investigated comparatively recently (Broom, 2011). Definitions of animal welfare, and public interest in the welfare of farmed animals has evolved along with changing moral and ethical viewpoints regarding the treatment of animals in society (Cornish, et al., 2016; Fisher, 2009; Fernandes, *et al.,* 2019; Alonso, et al., 2020).

An early concept of animal welfare assessment was the five freedoms, established in 1979 by the Farm Animal Welfare Council in response to the Brambell Committee report on the welfare of farm animals in intensive rearing systems (Brambell, 1965; FAWC, 1979). These five freedoms address the concept of animal emotions, stating that animal welfare can be promoted by providing an animal with the following freedoms: 1) freedom from hunger and thirst, 2) freedom from discomfort, 3) freedom from pain, injury and disease, 4) freedom to express normal behaviour and 5) freedom from fear and distress. The five freedoms are the foundation of the five needs, which are embedded within UK legislation, that outline the responsibilities of animal owners to ensure the welfare of animals within their care (Animal Welfare Act , 2006).

As animal welfare became established as a scientific discipline in the late 1980s and early 1990s, the concept of animal welfare was defined to reflect the idea that an animal's welfare state could be measured, as reviewed by Fraser (2008) and Broom, (2011).

During this time, different views and values emerged concerning the management of farm animals and how their welfare should be evaluated (Duncan & Fraser, 1997; Fraser, et al., 1997). These main societal views are further described by Fraser (2003) and relate to 1) views that emphasise biological functioning, health, growth and productivity 2) views that emphasise animal feelings and emotions and 3) the view animals should be able to live as naturally as possible. Different priorities and values may lead to different conclusions on what constitutes acceptable levels of animal welfare and what actions need to be taken to safeguard and improve it (Fraser, 2003).

These differing values also led to some debate as to whether animal welfare is wholly about what animals feel (Duncan & Petherick, 1991; Duncan, 1993) or predemoninatly about animal health (Husu-Kallio, 2008; Broom, 2011). More commonly, animal feelings were considered a central issue in welfare science, but that health aspects should also be considered important (Dawkins, 1980; Dawkins, 1990).

While the five freedoms are outcome measures that acknowledge both an animals health status and the concept of animal feelings (Webster, 2016), they focus on the absence of suffering, rather than the presence of positive welfare states (FAWC, 2009 ; Mellor, 2016) and describe a snapshot of welfare at a moment in time rather than the causes and consequences of stressors over the lifetime of an animal (Webster, 2016).

Within the framework of the five freedoms, any negative emotional states and environmental challenges can be interpreted as detrimental to welfare. However, freedom from negative emotional states at all times may not only be unobtainable but undesirable; as a certain level of environmental challenge may be necessary to provide animals with a broad predictive physiological and behavioural capacity to anticipate environmental challenges (McEwen, 1998; Korte, *et al.*, 2005; Korte, *et al.*, 2007).

Broom (1986) defined the welfare of an individual animal as "it's state as regards its attempts to cope with its environment", based on how an animal reacts with behavioural and physiological feedback mechanisms to maintain homeostasis. Evidence of these coping strategies can then be measured to determine how well an animal is coping with its environment and make judgments about an animal's welfare on a continuum from "very poor" to "very good".

Korte *et al*. (2007) later proposed an animal's welfare can be measured by quantifying allostatic load, which further recognises the negative impacts of hypostimulation and the need for appropriate environmental challenge. In comparison to homeostasis, allostasis considers not only the mechanisms

animals use to respond to environmental challenges, but also their needs to meet anticipated future demands.

Korte *et al.* (2007) suggests the five freedoms are more appropriate as an ethical framework to approach animal welfare, rather than a science-based criterion for welfare assessment. Despite this, the five freedoms are internationally recognised and form the guiding principles of the World Organisation for Animal Health (OIE) which defines animal welfare as " the physical and mental state of an animal in relation to the conditions in which it lives and dies" (OIE, 2019).

While phyical and mental health are both considered as part of human and animal welfare, they are frequently disscussed seperatly. For example, Dawkins (2003) proposes that measures of animal welfare can viewed within the context of two key questions; 1) is the animal healthy and 2) does the animal have what it wants.

The assessment of animal welfare has thus evolved to consider a diverse range of interdisciplinary variables. As there is no acceptable sole measure of animal welfare, a range of measures need to be considered in order to attempt to understand an animal's experience and how they are impacted by human management and control (Webster, 1998; Dawkins, 2006).

In veterinary and animal production fields, animal welfare assessment has traditionally focused on an animal's physical health, adequate resource provision and an animal's ability to cope with its environment (Broom, 1986; Broom, 1988). This is reflected in welfare assessment techniques and protocols for broiler chickens, such as the Welfare Quality® protocol, where welfare indictors may consist of both resource-based and animal-based measures (Bock & de Jong, 2010). Resource based measures include: space (stocking densities), litter provision, adequate ventilation, lighting and thermal control, which may serve to identify risk factors for poor welfare outcomes (Manning, *et al.*, 2007a; Granquist, et al., 2019). However, animal-based measures of welfare, such as gait assessment (Kestin, *et al.*, 2001), contact dermatitis (Berg

& Algers, 2004), and signs of thermal discomfort (McLean, *et al.,* 2002), are important to determine the consequences of husbandry and management aspects on the animal itself and the animal's experience (EFSA, 2012).

In order for an animal-based measure to be considered a good welfare indicator it should be robust (parameters relating to intra and inter observer reliability, re-test reliability and repeatability of the measure) and valid (evidence should support that the measure is reflecting the construct it was designed to measure) (Taylor & Mills, 2006; EFSA, 2012).

Animal based welfare indicators can also be viewed in the context of "lead" and "lag" indictors (Manning *et al.*, 2007a). Lag indictors refer to measures that are examined at the end of the growing cycle obtained from the farm or processing companies, such as: total flock mortality, numbers of leg culls, birds dead on arrival and condemnations at slaughter. While such indictors may offer retrospective information about an animal's welfare, the measure can only inform future management. In contrast, lead indicators are intra-cycle measures that provide information on welfare status in time for preventative or corrective action to be taken within the growing cycle. For example, water consumption has been identified as a key "lead" indicator relating to bird stressors, feed and water quality issues and problems with the animal's environment (Manning *et al.,* 2007a; Manning *et al.,* 2007b).

There is growing pressure for producers to improve welfare standards. Animal welfare can be considered a public good, with clear links to wider economic and societal concerns such as public health and environmental health (FAWC, 2011). Broom (2010) argues that " No system or procedure is sustainable if a substantial proportion of people find aspects of it now, or of its consequences in the future, morally unacceptable".

Sustainability refers to "meeting present needs without compromising the ability of future generations to meet their needs" (WCED, 1987), and encompasses economic, environmental and social aspects. Improving the sustainability of animal production, is a priority for sustainable development

with considerable scope for mitigation in terms of economic and environmental sustainability (Poore & Nemecek, 2018) and social sustainability (Cornish, *et al.,* 2016).

Further challenges arise in animal production systems when different aspects of sustainability are in conflict. For example, conflicts may arise due to pressure on farmers to produce competitively priced animal products as efficiently as possible under small financial margins, which can create barriers to the adoption of husbandry improvements that promote good welfare (Gocsik, *et al.*, 2013). Different production systems also present different challenges, as demonstrated by Gocsik *et al* (2016) who evaluated the cost-efficiency of different broiler production systems along with assessments of animal welfare using the Welfare Quality® Protocol. While welfare was sharply increased in alternative systems compared to conventional broiler production, production costs increased by 23-139%. Producers may be less likely to swap from conventional farming methods if the financial risks are greater.

Farmers may also underestimate the impacts of welfare concerns such as lameness, which limits the uptake of management practices shown to reduce this issue despite the potential for added economic benefits (Gocsik, *et al.*, 2017).

Additionally, while consumers may indicate concern for broiler welfare (Hall & Sandilands, 2007), and demonstrate willingness to pay for products from animals reared to higher welfare standards (Mulder & Zomer, 2017), there remains a considerable knowledge gap and lack of public understanding concerning broiler chicken production systems and which husbandry and management modifications changes are the highest priority for improving welfare (de Jonge & van Trijp, 2013; Lusk, 2018).

Solutions to these conflicts may emerge through attempting to quantify the ways that good animal welfare contributes to more efficient farming; for example: improved health and reduced mortality, improvements in product quality, improvements to disease resistance and reductions in the use of

medication, lowered risk of zoonotic disease transmission and increased job satisfaction of farmers and stockmen (Dawkins, 2016; Broom, 2019).

Just as aspects of animal welfare assessment can be defined within the context of two questions 1) are animals healthy and 2) do they have what they want (Dawkins 2003), efforts to improve the welfare of production animals can arguably be simplified in to two main objectives: a) implementing evidence-based changes to animal management and husbandry or b) the development and validation of robust welfare assessment indicators, tools and techniques (figure V.II).

The work presented in this thesis explores aspects of these objectives. The acceptance and successful implementation of husbandry changes may be influenced by a number of factors reviewed above including: economic constraints, sustainability goals (particularly if a proposed change to improve welfare also results in increased energy consumption per unit of output), government legislation and other specifications and protocols imposed by processing companies and retailers, consumer perceptions and demands and additionally farm owner and staff attitudes to welfare (such as the impact and priorities of welfare concerns as perceived by the farmer and farm staff).

Multiple welfare measures need to be considered to determine animal welfare status. Data may be collected by the farmer, auditing companies or academic or industry-based researchers to evaluate current practice. Robust and informative welfare assessment indicators and protocols are essential to highlight areas for improvement in current practice, in addition to monitoring any implemented changes to management or husbandry procedures to determine their efficacy. Welfare assessment methods too are influenced by the factors discussed above and must also adapt to collective understandings and definitions of animal welfare.

The current study will investigate a potential husbandry refinement for broiler chickens. The impact of supplementary ultraviolet wavelengths on indoor reared broiler chickens will be explored, and the value of ultraviolet

wavelengths for improving aspects of broiler chicken performance, health welfare will be investigated.

Qualities of lighting are known to be important for welfare in a range of species (McLennan & Taylor-Jeffs, 2004; Migaud, et al., 2007; Oliveira & Lara, 2016; Taylor, et al., 2006). Aspects of the lighting environment found to be important for welfare include: the length of the photoperiod (day-night cycle), light intensity and the wavelength composition of the light source (Campo & Davila, 2002; Campo, et al., 2007; Deep, et al., 2013; Fuller, et al., 2016).

The importance of lighting conditions for poultry and how they can be optimised is an area of research that is beginning to be explored more thoroughly but has not yet been studied as extensively as other elements of poultry management. This is surprising, considering the clear importance of vision to birds and thus its potential for improving their welfare.

Secondly the associations between selected welfare, health and performance indicators will be explored.
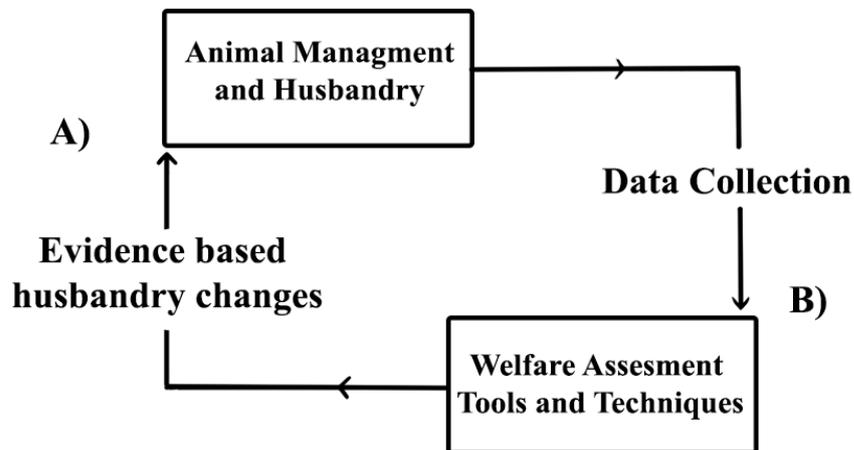
Figure V.II -Improving the welfare of production animals

Efforts to improve animal welfare can be simplified into two main objectives; A) making evidence-based changes to animal management and husbandry practices, and B) developing and validating tools for welfare assessment. The success of both of these objectives are influenced by a number of factors including: economic constraints, sustainability goals (particularly if a proposed change to improve welfare also results in increased energy consumption per unit of output), government legislation and other specifications and protocols imposed by processing companies and retailers, consumer perceptions and demands and additionally farm owner and staff attitudes. The objectives are represented as a cycle as robust and informative welfare assessment indicators are essential to highlight areas for improvement in current practice and for ongoing monitoring of implemented changes.

V. III) THE BIOLOGICAL IMPACTS OF LIGHT

Light influences the behavioural and physiological activities of poultry by two main mechanisms. Firstly, retinal photoreception in the eye facilitates vision and visually guided behaviours. Secondly, light acts to regulate the endocrine system and maintain circadian and circannual rhythms through both retinal and extra-retinal receptors, allowing for the synchronisation of many essential functions including the regulation of body temperature, feeding and digestion and the activity of endocrine pathways essential for growth, sexual maturation, and reproduction (Olanrewaju, *et al.,* 2006).

Circadian rhythms are an essential feature regulating the behavioural and physiological organisation in higher organisms including birds (Gwinner & Brandstätter, 2001). Endogenous oscillating molecular pathways generated within cells form the biochemical basis of circadian rhythms, which synchronise over a 24-hour period (Dunlap, 1999). A distinctive feature of circadian rhythms is that they persist in constant darkness but become strongly entrained to fluctuations in photopic conditions (Zawilska, *et al*., 2004).

The avian biological clock consists of three key components that interact jointly to regulate stable behavioural and physiological rhythms: the retina, the pineal gland (located between the cerebral hemispheres at the top of the brain) and the hypothalamic oscillator or deep encephalic photoreceptor, which is the functional equivalent of the mammalian hypothalamic suprachiasmatic nuclei (SNC) (Gwinner & Brandstätter, 2001) .

Light entering the eye stimulates the synthesis and release of dopamine, which suppresses melatonin production by reducing the activity of the enzyme arylalkylamine N-acetyl-transferase (AANAT), which is the main enzyme involved in melatonin production in the retina (Morgan, et al., 1995). Dopaminergic neurotransmission also suppresses the activity of ANNAT and thus the synthesis of melatonin in the pineal gland (Zawilska, *et al.*, 2004), resulting in a light dependent rhythmic release of melatonin, with high levels

of melatonin during the night and low levels during the day. Melatonin regulates both daily and seasonal physiological rhythms including the maintenance of sleep-wake cycles, thermoregulation, locomotory activity patterns, and the cardiopulmonary, reproductive, excretory, and immunomodulatory organ systems in birds (Apeldoorn, *et al.*, 1999; Gwinner, *et al.*, 1997). Alteration of photoperiods or exposure to light during the dark phase of the photoperiod alters melatonin synthesis and can induce a "phase-shift" or "phase reversal" in domestic fowl (Cain & Wilson, 1974; Csernus, 2006; Faluhelyi & Csernus, 2007).

Unlike mammals, the pineal gland of birds is directly photosensitive, with light activation via the skull and cranial tissues demonstrably suppressing melatonin production (Csernus, 2006; Faluhelyi & Csernus, 2007). The pineal gland is able to regulate melatonin synthesis independently of retinal photoreception, as demonstrated by *in vitro* studies of the pineal organ (Deguchi, 1979) and observations of chicks that had been surgically blinded yet were still able to entrain to light-dark cycles (Nyce & Binkley, 1977). The retina too is capable of acting as an independent circadian oscillator in response to light after pinealectomy (Bian, *et al.*, 2019).

Like the pineal gland, the hypothalamic receptors of birds respond directly to light that has passed through the skull, and to neural signals from the retina and the pituitary gland via a polysynaptic neural pathway (Gwinner & Brandstätter, 2001). Thus, light is an important environmental cue regulating activation of the hypothalamic pituitary gonadal (HPG) axis. Light that reaches the deep encephalic receptor regulates the release of gonadotrophin releasing hormone (GnRH), which stimulates the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the pituitary gland; regulating sexual maturation and reproductive function.

Different wavelengths of light vary in their efficiency for eliciting a photosexual response via stimulation of the hypothalamus. Shorter wavelengths of light are more readily absorbed by surrounding cranial tissues than longer wavelengths of light, with wavelengths below 430nm not penetrating cranial tissues at all

(Hartwig & Veen, 1979).   Even direct stimulation of the hypothalamus using optic fibres required 23 times as much violet light (470nm) compared to red light (650nm) to induce a comparable rise in LH concentrations in quail (Foster & Follett, 1985).

In contrast, shorter wavelengths are more efficient at influencing pineal activity than longer wavelengths in mammals (Cardinali, *et al.,* 1972), and birds (Csernus, *et al.,* 1999) due to the presence of a photoreceptive molecule (pinopsin) which is responsive to blue light in the domestic fowl (Okano, *et al.,* 1994). Rhythmic secretion of melatonin from the chicken pineal gland and the retina is impacted by the light spectra. Retinal illumination with UVA during the dark phase of the photoperiod produces a phase shift in circadian osscilators through dramatically decreasing the action of AANAT and melatonin systhesis in the pineal gland and the retina. This occurs via the stimulation of retinal N-methyl-D-aspartate (NMDA) glutamate receptors rather than the Dopaminergic neurotransmission associated with the retinal reception of white light (Rosiak & Zawilska, 2005).

Jin, *et al.* (2011) investigated the impacts of blue, green, red and white light on AANAT activity and serum melatonin levels in broilers. All light treatments resulted in the maintenance of circadian rhythms, though the amplitude of plasma melatonin was increased in the green light treatment compared to other treatments due to the promotion of AANAT expression in the retina and pineal gland (Jin, *et al,.* 2011). Different light colours alter the expression of circadian clock genes in both the pineal gland (Jiang, *et al.,* 2016) and the retina (Bian, *et al.,* 2020; Cao, *et al.,* 2017).

Overall, the photoperiod (Faluhelyi & Csernus, 2007), light intensity (Zawilska, *et al.,* 2004), and light spectra (Jin, *et al.,* 2011) all influence the regulation of melatonin production and other endocrine biorhythms (Apeldoorn, *et al.,* 1999; Gwinner, *et al.,* 1997). It is therefore important to consider the impacts of lighting regimes used in the captive management of broilers, which may impose light conditions that result in the inappropriate stimulation, or dysregulation of these essential biological systems.

For example, broilers were commonly reared under constant illumination to maximise feed intake and weight gain (Lewis & Morris, 2006). However, this leads to heightened levels of fear and stress (Zulkifli, *et al.*, 1998), increased susceptibility to heat stress and weakened immune responses; which can be alleviated with administration of melatonin (Abbas, *et al.,* 2007; Saito, *et al*., 2005; Nelson, *et al.*, 1994;), highlighting the importance of this rhythm in the maintenance of bird health and welfare.

Additionally, broilers are often reared under dim light conditions in order to reduce their activity levels and improve their feed conversion ratios (Olanrewaju, *et al.,* 2006; Proudfoot & Sefton, 1978). In such conditions, chickens will be more dependent on retinal photoreception for the maintenance of circadian rhythms due to the lower minimum thresholds of light intensity for pineal stimulation (Morgan, *et al*., 1995). In environments where the amplitude of the light: dark cycle was lower (5 lux daytime illumination), broilers showed less behavioural synchrony and had more frequent but shorter bouts of rest compared to broilers reared under conditions where there was a greater amplitude of the light dark cycle (200 lux daytime illumination) (Alvino, *et al*., 2009b). Sleep is crucial for energy conservation, tissue restoration and growth as well as supporting normal cognitive function (Blokhuis, 1983). Thus, light environments that do not facilitate rest and normal circadian rhythms are detrimental to the health and welfare of broiler chickens (Olanrewaju, *et al.*, 2006).

V. IV) THE VISUAL SYSTEM OF POULTRY

Vision is the dominant sense for many birds, which utilise some of the most complex retinas of any vertebrate in order to obtain information about their surroundings (Walls, 1942). Birds have very large eyes relative to their body size and the eyes of the domestic chicken weigh approximately the same as its brain, making it no exception (Appleby, *et al.*, 1992). However, only a small portion of the corneal surface is visible, concealing the fact that the eyes may occupy 0.5 or more of a bird's cranial capacity (compared to 0.05 in humans) (McLelland, 1990; Waldvogel, 1990; Brooke*, et al.,* 1999).

The eye is protected by mobile upper and lower eyelids and a nictitating membrane which moves laterally across the eyeball (figure V.IV.a).
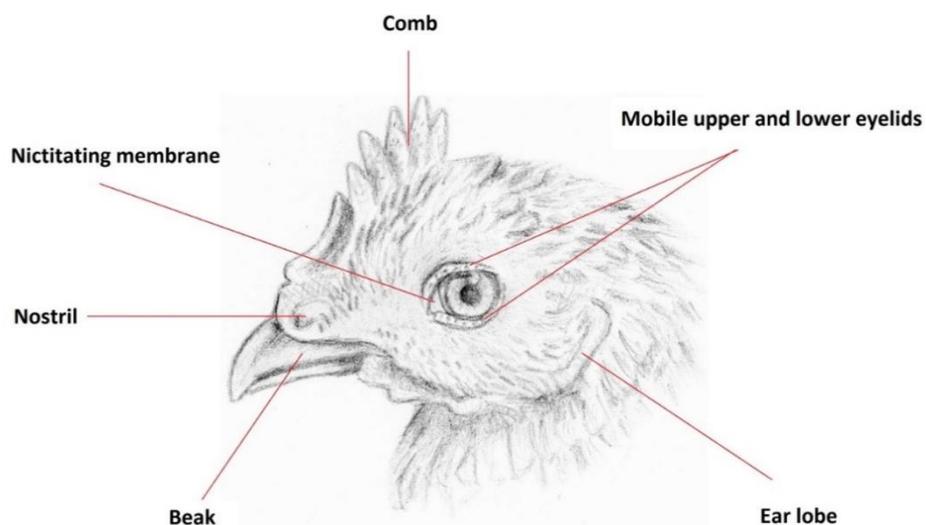
Figure V.IV.a- External features of the adult domestic chicken head, *(Charlotte James 2014) Annotated with information from McLelland (1990).*

As light enters the eye of a chicken it passes through the cornea, anterior chamber, lens and the vitreous chamber, which together act to focus and magnify an inverted image on to the light sensitive neural tissue of the retina (figure V.IV.b). The amount of light entering the eye is controlled by the coloured Iris, the movements of which alter the size of the pupil. Birds have relatively large pupils which can be rapidly adjusted in size allowing for precise control of image quality (Waldvogel, 1990).
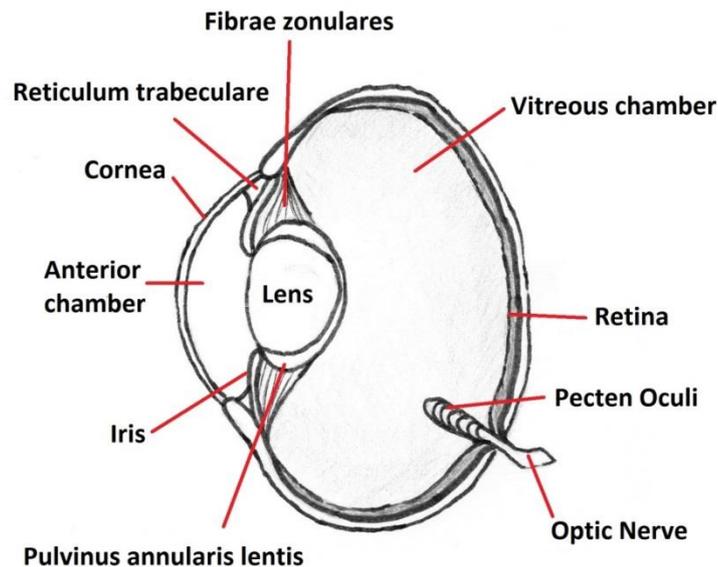
Figure V.IV.b- Cross section of the internal anatomy of the domestic chicken eye (Charlotte James, 2014) adapted from Poultry CRC (2006). Many structures are similar to the mammalian eye with the exception of the pecten. The pecten is a highly vascularised pleated structure which provides the avascular retina with nutrition by diffusion through the vitreous body (McLelland, 1990; Waldvogel, 1990).

Only photons that are absorbed by the photoreceptors can be used for vision. Therefore, the spectral sensitivity, relative abundance, arrangement, and types of different photoreceptors partly define the visual capabilities of an animal (Hart, 2001). However, visual perception involves both the evaluation of visual stimuli and attention to and differential processing of selected information by the brain to determine a behavioural response. Therefore, both the underlying mechanisms of visual processing and the cognitive abilities of animals must be considered to attempt to understand how visual scenes are processed and perceived (Knudsen, 2020).

Prescott *et al.* (2003) proposed that the most important visual abilities of the chicken that relate to their management in commercial conditions include their spectral sensitivity, flicker sensitivity, accommodative range and visual acuity, as these qualities primarily determine how the animal perceives its environment and thus how light will impact visually mediated behaviours.

Visually mediated behaviours include finding sources of food and water, locating suitable nest sites, scratching, identifying safe places to roost and the recognition of conspecifics.

Hens show the ability to discriminate between individual group mates when kept in small groups under environmental and experimental conditions that facilitate transmission and perception of social cues, (Abeyesinghe, *et al.*, 2009), with vision thought to essential in this regard.

This raises concerns that the conditions in which chickens are reared under commercial settings may disrupt visual social signals potentially impacting bird welfare (D'Eath & Stone, 1999; Prescott *et al.*, 2003).

This is further evidenced by studies where hens failed to discriminate between familiar and unfamiliar birds in situations where visual cues were compromised; for example, where only non-visual social signals were available (Hauser & Huber-Eicher, 2004), when light intensity was 1 lux (Kristensen, *et al.*, 2009) or under coloured light treatments (D'Eath & Stone, 1999).

However, other factors, such as group sizes may also influence the social behaviour of domestic chickens. Hens kept in larger group sizes are less able to identify familiar and unfamiliar birds than those kept in small groups (D'Eath & Keeling, 2003), and both laying hens and broilers housed in larger group sizes have been shown to display social tolerance and reduced agnostic behaviours when housed in larger group sizes (Estevez, *et al.*, 1997; D'Eath & Keeling, 2003).

Avian eyes are less spherical than mammalian eyes and can be categorised as flattened, globose or tubular reflecting their different visual adaptations (Waldvogel, 1990). Chickens have a "flattened" eye which gives them a wider field of view but sacrifices some resolution.

The wide visual field of the domestic chicken is also aided by the lateral position of the eyes which gives them a visual field of 330° (Appleby, *et al.,* 1992), though only 26° of this is covered by binocular vision (Poultry CRC, 2006), (figure V.IV.c). Chickens exhibit lateralization of hemispheric functions and primarily use their right eye for perception of smaller details such as food (Rogers, 1997), and their left eye for observation of novel stimuli and predator vigilance (Dharmaretnam & Rogers, 2005) and for distinguishing between familiar and unfamiliar conspecifics (Vallortigara & Andrew, 1994).
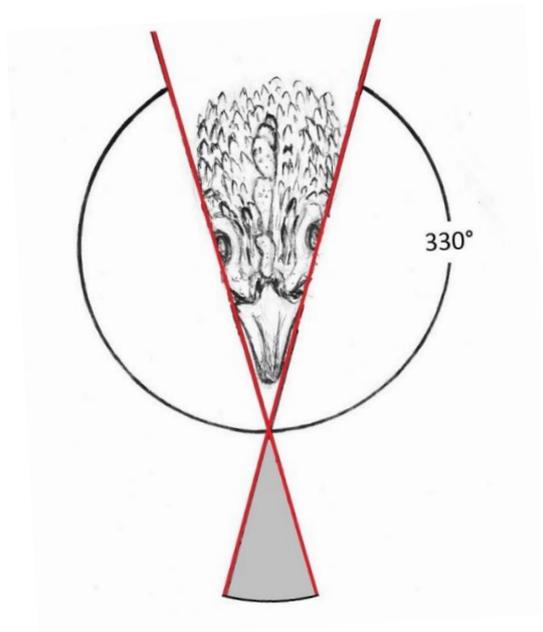
Figure V.IV.c - The visual field of the domestic chicken
(Charlotte James 2014) adapted from Appleby, et al., (1992). Binocular vision is indicated in grey and covers 26° of the total visual field.

The short range of lower frontal binocular vision is further involved in focusing when pecking food items and the recognition of familiar and unfamiliar conspecifics, though evidence suggests they are unable to make this discrimination unless they are within 8-30cm from the other bird (Dawkins, 1995).

This is consistent with further investigations by Jarvis, *et al.* (2009) where psychophysical operant experiments showed that chickens have relativley poor spatial contrast senstivity compared to humans. The spatial contrast sensitivity function (CSF) relates to the ability of the visual system to distinugish bright and dim components of a static image, wheras visual acuity relates to the angle at which an idividual can resolve two separate points. Sine wave gratings consisting of parallel bars of variying contrast and spatial frequency have be used to assess the CSF of humans and chickens (Gover et al., 2009; Jarvis et al.,2009). Mathmatical modelling indicated that to an observing chicken,

maximum visibility of another chickens comb would occur at a viewing distance of 200 mm, while at a distance of 1600 mm another chicken's comb would be outside the chickens range of spatial sensitivity (Jarvis et al.,2009). These calculated values align well with prefered viewing distances for social recognition in other behavioural studies (Dawkins 1995; Dawkins & Woodington, 1997).

Spatial contrast senstivitiy is also influenced by light intensity (the stimulus luminance), as demonstrated by Gover *et al.* (2009), where the CSF of chickens was investigated under stimulus luminances from 0.06 - 57.35 cd m^{-2}. Over the range of photopic vision (1.79–57.35 cd m^{-2}), the change in acuity was less for chickens (1%) compared to humans (32%), suggesting an adaption in the domestic fowl to dim lighting conditons. Therefore, Gover et al. (2009) suggested that the spatial visual ability of chickens were unlikely to be compramised in the dim conditions of poultry housing; which must be a minimum of 10 lux for laying hens (DEFRA, 2018b) and 20 lux for broilers (DEFRA, 2018). This is in agreement with the findings of Kristensen, *et al*., (2009) where only the dimmest light intensity of 1 lux was thought to have affected visual aspects of social communication.

Chickens hatch from the egg with well-developed vision, with all subtypes of photoreceptor distinguishable from day 19 of incubation (Wai, *et al.*, 2006).

While the general organisation and cell types found in the retina of the chicken are similar to those found in the mammalian eye, birds have many distinct features of their visual systems that differ from humans.

Both humans and birds possess rod cells, which are specialized for vision in low light or scotopic conditions and cone cells which are for daytime or photopic vision and respond to specific wavelengths of light, enabling colour vision. Birds, including the domestic chicken, also possess double cone cells which are thought to be involved in the detection of movement (Hart, 2001), though recent studies have not found evidence for this in the domestic chicken (Rubene, et al., 2010).

The spectral sensitivity of the cones of the domestic chicken can be estimated based on results of Microspectophotometry (Bowmaker, *et al.*, 1997) and spectrophotometry of retinal extracts or *in vitro* regenerated visual pigments (Wald, 1937; Bliss, 1946; Fager & Fager, 1981; Fager & Fager, 1982; Okano, *et al.,* 1989; Shichida, *et al.,* 1990; Wald, *et al.,* 1955; Yokoyama, *et al.,* 2000). There are four distinct types of single cone; Ultraviolet or Violet sensitive (UVS/VS), short wave sensitive (SWS), medium wave sensitive (MWS) and long wave sensitive (LWS) which have different wavelength responses resulting in tetrachromatic colour vision (Table V.IV). However, while *In vitro* methodologies implies the existence of colour vision, results of studies may vary due to noise in data or different levels of accuracy in wavelength calibration between studies (Hart, 2001). Behavioural experiments showing evidence of colour discrimination are necessary to demonstrate what the animal itself perceives, and to consider the visual system and visual processing mechanisms as an integrated unit (Prescott & Wathes, 1999;Goldsmith, 2006).

Table V.IV. Wavelengths of maximum absorbance (λmax) of the four cone types obtained from the domestic chicken
The four types of single cone are Ultraviolet or Violet sensitive (UVS/VS), short wave sensitive (SWS), medium wave sensitive (MWS) and long wave sensitive (LWS)

Method used to determine (λmax)	Visual pigment λmax (nm)				
	UVS/VS	SWS	MWS	LWS	Rod
microspectrophotometry	419	455	508	570	506
spectrophotometry of retinal extracts or in vitro regenerated visual pigments	415–425	449–455	508	560–571	500–504

Behavioural experiments demonstrate that chickens have well developed colour vision and spectral sensitivity that is distinct from humans (Prescott & Wathes,1999). Humans have three distinct cones which allow perception of colours in the violet/blue (450nm), green (550nm) and red (700nm) parts of the spectrum. In comparison, chickens are more sensitive to light between 400-480nm and 580-700nm and can perceive UVA wavelengths as low as 360nm (Prescott & Wathes, 1999; Lewis & Morris, 2006: Figure V.IV.d). UV vision is further facilitated by the ocular media of birds which, unlike humans, is able to transmit UV wavelengths (Bennett & Cuthills, 1994; Osorio, et al., 1999; Lind, *et al.*, 2014).
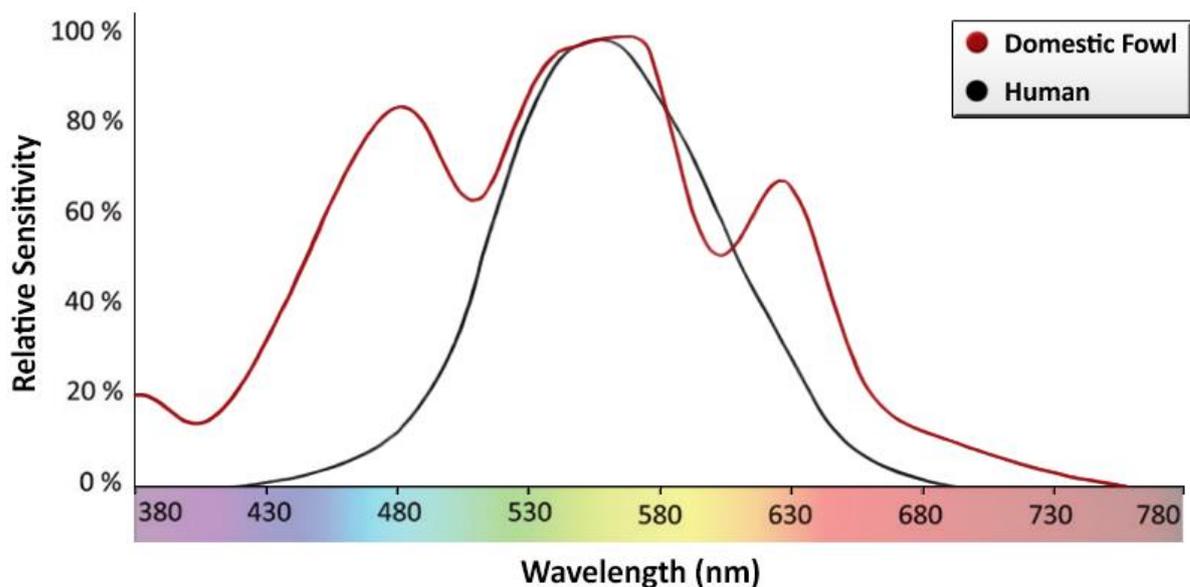
Figure V.IV.d - Comparison of the relative spectral sensitivities of the domestic fowl *(normalised to a sensitivity of 1.0 at 565nm) and humans (normalised to a sensitivity of 1.0 at 555nm). Data from Prescott and Wathes (1999).*

In addition to four distinct cone receptors, chickens also possess red, yellow, almost colourless, and transparent oil droplets within their cone cells (Walls, 1942; Goldsmith, *et al.,* 1984). These 4 pigmented cone oil droplets contain varying amounts of carotenoids which act as long-pass filters, removing short

wavelengths of light and improving the bird's ability to distinguish between colours (Partridge, 1989; Hart, 2001; Goldsmith, 2006).

Flicker sensitivity is another important feature of the avian visual system which is related to the speed birds are able to process temporally varying visual stimuli (temporal resolution). This is typically determined using behavioural experiments to investigate critical flicker fusion frequency (CFF) (Jones, *et al.,* 2007), which is the frequency at which a flickering light source appears as steady or continuous at a given light intensity (Landis & Hamwi, 1954).

CFF may be influenced by light intensity (Jarvis, *et al.,* 2002; Lisney, et al., 2011) age (Brozek & Keys, 1945; Brundrett, 1974), and the spectral composition of the light source (Nuboer, *et al.,* 1992a; Rubene, *et al.,* 2010).

Jarvis et al. (2002) found mature laying hens were less sensitive to flicker bettween luminance levels of 10-1000 cd/m^2 and over the same ranges their mean CFF's ranged from 39.2Hz- 71.5Hz which were similar or slightly greater than that of humans (which ranged from 40.8 - 58.2 Hz) depending on the illuminance. However,the light stimulus used in this study did not contain UVA which may have compramised the chickens abilities.

Rubene *et al.* (2010) used an operant training task to determine the CFF of chickens using UVA only, full spectrum (including UVA), white UV-free and amber Light Emitting Diodes (LED) at four different light intensities with a luminance of 120-800 cd/m^2. Both light intensity and the light wavelength were found to impact the CFF of chickens, which ranged between 44 - 83 Hz, with the highest values observed in the UVA light test condition, suggesting UVA light improves the temporal resolution of chicken vision. Nuboer, *et al.,* (1992a) reported a CFF of up to 105Hz for a flickering blue light source compared to 80 Hz for a white compact fluorecent light, which also suggests that the CFF of chickens is influenced by the spectral composition of the light source.

Lisney *et al.* (2011) tested CFF over a wider range of light intensities than previous studies from a luminance of 0.2 - 2812 cd/m^2 with a full spectrum light stimulus containing UVA, recording slightly higher CFF's of in an old Swedish

game breed of chicken ranging from 19.8 - 87 Hz , though individual birds were capable of perceiving a flicker of 100Hz at an illuminance of 1375 cd/m^2 . These slightly higher values could also have been influenced by the breed selected for this study.

Collectively the liturature reviewed above suggests that under commerical conditions (which are typically quite dim) chickens will not percive the flicker of low frequency fluorescent lights (100 or 120 Hz). However, (Boshouwers & Nicaise, 1992), found broilers exposed to low frequency fluorescent lighting were less active than those exposed to high frequency fluorescent lighting. Additionally, while behavioural tests show chickens may not consciously perceive flicker above 100Hz , electroretinograms of commercial laying hen strains obtained CFFs of 118–119 Hz, indicating that the retina itself is capable of responding to higher flicker rates even if this information is not consciously perceived (Lisney, *et al*., 2012). In human's and non-human animals' exposure to "invisible flicker" has been linked to headaches, visual strain, neurological effects and physiological or behavioural stress (Inger, *et al*., 2014). Therefore, further investigation of the potential impacts of flicker on welfare may still be warranted (Lisney, *et al*., 2012).

Furthermore, the majority of studies investigating the CFF of chickens have focused on mature hens (Jarvis, *et al.,* 2002; Lisney, et al., 2011; Nuboer, *et al.,* 1992a; Rubene, *et al.,* 2010), Therefore, as age and strain is thought to influence CFF, further studies on broiler chicken strains at commerically relavant ages would be important before the flickering of artificial lightsources is discounted completely as a potential stressor to broiler chicken welfare.

The literature reviewed in this chapter highlights the importance of considering the visual capabilities of broilers when developing and implementing lighting regimes for broiler production.

V. V) THE IMPACTS OF LIGHTING ON PERFORMANCE HEALTH AND WELFARE IN BROILER CHICKENS

Despite notable differences in the visual systems of the domestic chicken compared to humans, broiler chickens are typically kept in lighting conditions deemed acceptable by human standards. The specifics of poultry vision and the impacts of light on aspects of their behaviour and physiology should be considered to ensure lighting programmes are not detrimental to the health and wellbeing of broiler chickens, support the development of normal vision and allow them to see well enough to carry out critical visual tasks (Prescott, *et al.,* 2003).

There three main lighting parameters that determine the light environment of a poultry shed include; light intensity, photoperiod, and the composition of wavelengths or "colour" of the light. These parameters effect performance (Lewis, et al., 2009; Olanrewaju, et al., 2018), health (Blatchford, et al., 2009), and welfare (Prescott, et al., 2003; Schwean-Lardner, et al., 2013) of broilers, though the impacts of lighting on different aspects of health and welfare overlap greatly (figure V.V).
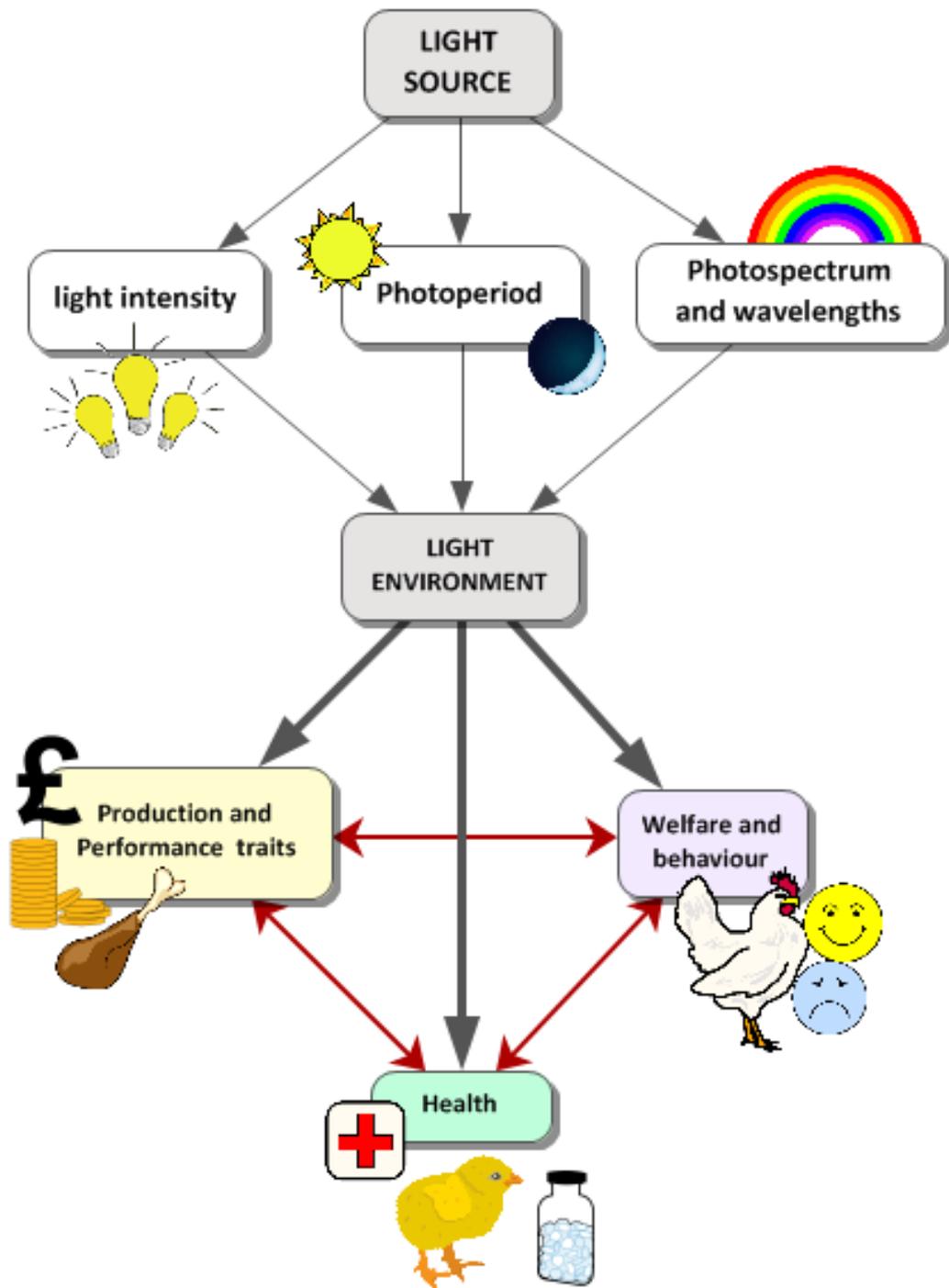
Figure V.V - Summary of the main areas of interests for research in to poultry lighting
The choice of light source and the lighting parameters affect the quality of the light environment. These parameters effect performance (Lewis, et al., 2009; Olanrewaju, et al., 2018), health (Blatchford, et al., 2009), and welfare traits (Prescott, et al., 2003; Schwean-Lardner, et al., 2013). Red double headed arrows indicate how these three key areas overlap greatly. For example, healthy pain free birds will likely have better standards of welfare and perform better (Dawkins, 2016).

The lux unit is routinely used to measure light intensity in poultry sheds. However, this value is based on the spectral sensitivities of humans and is not appropriate for measuring the quality of the light environment from the perspective of a chicken. Both the yellow- red (580-700nm) and the violet-blue (400-480) parts of the spectrum are perceived as brighter by the domestic fowl than they are by humans (Prescott & Wathes, 1999), meaning incandescent and fluorescent light sources that approximate white light to humans may be perceived as coloured light by the chicken (Nuboer, et al., 1992b). In addition to this, lux readings taken from barns with different light sources will not be comparable, due to their different spectral compositions (Prescott, *et al.*, 2003). Instead Nuboer, *et al.,* (1992b) and Prescott and Wathes (1999) describe the illuminance perceived by poultry using the Gallilux or clux unit (equation VI.IV).

$$I = (w.\ s.\ 683) / (4\pi.d^2)$$

Equation V.V) The illuminance perceived by the domestic chicken (clux or gallilux)
 *at a given distance from a light source in meters (**d**) is obtained by integrating the illuminance (**I**) for 5nm segments of the spectrum. **w** is the power output of the lamp (W) in 5nm segments and **s** is the relative sensitivity of the domestic fowl. The maximum luminous efficacy for human photopic vision is 683 lumens/W, which has been found to be acceptable to use in this equation (Saunders, et al., 2008).*

Using equation V.V (at a distance of 1.5m away from a light source, and at a reflectance value of 0.2) a 15W incandescent lamp would perceived by a human as 5.6 lux compared to 8.1 gallilux for the domestic fowl (Lewis & Morris, 2006). Greater differences are seen where the light composition includes more short or long wavelengths. For example, a 36W blue fluorescent tube is perceived by humans as 37.8 lux compared to 196.8 gallilux for the domestic fowl (Lewis & Morris, 2006).

As a result of the distinct spectral sensitivity of poultry, many older studies investigating the effects of light intensity or wavelength may have inadvertently confounded their experiments by not taking the bird's spectral sensitivity into account (Prescott & Wathes, 1999; Lewis & Morris, 2000). In addition to this, when white light is dimmed using voltage reduction equipment it increases the proportion of red light emitted, which will still appear quite bright to the birds, leading to abnormal and variable experimental results (Lewis & Morris, 2006).

Studies on the effects of light intensity on broilers have demonstrated no effects on overall mortality or the incidence of leg disorders or Sudden Death Syndrome (Lewis & Morris, 2006). However, a meta-analysis of a series of experiments indicates a slight depression in growth rates, feed intake and a less favourable feed conversion ratio as light intensity increases between 1 and 100 lux (Newberry, *et al.,* 1986; Newberry, *et al.,* 1988; Charles, *et al.,* 1992; Lewis & Morris, 2006).

This reduction in growth rate may reflect the observation that higher light intensities have been found to encourage day-time activity in broilers (Newberry*, et al.,* 1988; Blatchford, *et al.,* 2009), which show preferences for higher intensities of 200 lux at 2 weeks of age and lower intensities of 6 lux by 6 weeks (Davis, *et al.,* 1999) .

Light intensity can also be altered to create dawn and dusk dimming periods that better replicate a natural photoperiod. This facilitates crepuscular feeding as the chickens fill their crops before the anticipated dark period, increasing their feed intake (Classen & Riddell, 1988; Savory, 1980). These periods of dimming are also thought to improve bird welfare through helping to regulate circadian rhythms and synchronising flock behaviours (Bryant, 1987; Lewis & Morris, 2006).

The photoperiod is also vital for the regulation of circadian rhythms and describes a period of illumination that is interpreted as "day" and a period of darkness or scotoperiod that is interpreted as "night". Conventional lighting programs for broiler chickens cycle every 24 hours. Unconventional programs

include Intermittent lighting programs, which have more than one period of darkness within a 24 hour cycle, and less commonly used ahemeral cycles, which have a single period of light and dark per cycle, though the cycle is longer or shorter than 24 hours.

Broilers have traditionally been maintained under almost continuous illumination. The long photoperiod was believed to maximises feed intake and growth of the birds. However, these lighting regimes are associated with higher incidences of Sudden Death Syndrome, leg disorders and higher overall mortality (Classen & Riddell, 1988; Lewis & Morris, 2006). A study by Schwean-Lardner *et al.,* (2013) concluded that 7 hours of consecutive darkness per 24-hour cycle promoted good health and welfare for broilers, based on a reduced incidence of metabolic diseases, improved mobility and better ocular health compared to broilers reared with shorter dark periods. Guidance provided by broiler producers discourages the use of continuous lighting except for within the first 24 hours after chick arrival and 1-3 days before final depletion (Aviagen, 2018; Cobb-Vantress, 2018).

Health problems may also be alleviated by starting broilers on 6-hour photoperiod until 21 days of age, which reduces their overall feed intake and body weight. The 6-hour photoperiod is then abruptly lengthened to 23-hours resulting in compensatory growth and similar final weights to birds kept under constant 23-hour illumination per 24-hour cycle (Lewis, 2001). The initial 6-hour photoperiods during development allow the bones and joints of the broiler to develop under less strain, reducing the incidences of leg disorders and overall mortality as the bird's skeleton is able to cope better with the subsequent compensatory growth.

Short cycle intermittent programs may also be introduced from 7 days of age to stimulate broilers to eat regular larger meals instead of an irregular intake of food or "grazing" throughout the day. The three most commonly used programs are; 8 cycles of 1 hour of light and 2 hours of dark 8 (1L:2D), 6 cycles of 1 hour of light and 3 hours of dark 6 (1L:3D) and 12 cycles of 0.25 hours of light and 1.75 hours of dark 12 (0.25L:1.75D). Males kept under these

conditions often attain similar or heavier finishing weights than birds kept on a 23-hour photoperiod (Buyse, *et al.,* 1996a; Buyse, *et al.,* 1996b). Weight gain following the switch to intermittent lighting programs is greater in males due to circulating testosterone levels, (Ohtani & Leeson, 2000) though both sexes tend to be less active and convert feed more efficiently on intermittent lighting programs (Kühn, *et al.,* 1996). The increased dark periods also lead to extended melatonin release which has a beneficial effect on immune homeostasis (Kliger, *et al.,* 2000).

The light intensity and photoperiod that broilers are exposed to is outlined in UK Legislation. Light intensity must be a minimum of 20 lux at bird eye level and follow a 24-hour rhythm cycle, with at least 6 hours of darkness, including an uninterrupted period of darkness of at least 4 hours excluding dimming periods (DEFRA, 2018).

However, despite the physiological and psychological impacts of wavelength composition on poultry there is little guidance available to direct farmers on which light sources and spectral compositions of light are most appropriate to support the performance, health, and welfare of broiler chickens.

Further research is therefore important to offer guidance, or even inform future legislation, stipulating preferable spectral compositions and light sources for broiler chickens that are economical and promote good welfare.

The subsequent chapters of this thesis will discuss the impacts of the spectral composition of the light environment on broiler chicken performance, health and welfare, particularly the impact of ultraviolet wavelengths.

The research presented in this thesis investigates the impact of three distinct spectral compositions including UVA only or both UVA and UVB wavelengths. The aim was to assess a range of performance, health and welfare indictors to determine if UV supplementation would be a valuable addition for commercially reared broiler chickens.

VI. GENERAL METHODS

VI. I) ANIMALS AND HUSBANDRY INFORMATION

The study undertaken at the University of Nottingham used 638 Ross 308 broiler chickens (PD Hook Hatcheries Limited, UK) obtained on hatch day. Chicks were from a 35-45-week-old parent flock and received vaccinations for Infectious Bronchitis at the hatchery. On arrival chicks were individually weighed and randomly assigned to one of six temperature-controlled rooms (n = 106-107 chicks per room/ n = 212-213 chicks per lighting treatment). Half the chicks arrived on the 24th of March 2015 (Flock 1, Room 1, 2 and 3) and remaining chicks arrived a week later on the 31st of March 2015 (Flock 2, Room 4, 5 and 6). Birds were fed *ad libitum* on a commercial five-part wheat-based diet provided by ABN, AB Agri, UK and reared on a bedding of wood shavings. Fresh bedding was added if the litter appeared wet. Each room had a small straw bale for enrichment purposes. The final stocking density reached by the end of the trial was a commercially representative $33kg/m^2$ based on a total useable area per room of $7m^2$ after subtracting space for feeders, drinkers and enrichment bales. All broilers were individually identified with wing tags at 7 days old.

All rooms were temperature controlled and set to follow a commercial heating and humidity program (provided by Frogmary Green Farm, Somerset). However, unforeseen problems with the environmental control of the animal unit's facilities were encountered throughout the study. Importantly, there were no reliable facilities in place for increasing humidity, meaning conditions were too dry, and some chickens were observed sneezing or had raspy breathing indicative of respiratory irritation. All rooms were affected by a ventilation failure on the 15th April which remained unreliable until the 6th of May. The target and mean recorded temperatures and humidity are listed in table VI.I. Standard biosecurity measures were in place governing entry of personnel. The experiment was reviewed and authorised by the Animal Welfare and Ethics Reviewing Body at the University of Nottingham.

Table VI.I – Mean daily recorded temperatures and humidity

Target commercial heat and humidity program and the mean daily recorded temperatures and humidity for all rooms, along with the standard deviation to show variation between rooms. The largest deviations from target temperature in the trial were on Day 1 in room 6. Temperature was 27.2˚C (4.9˚C below target) and on day 30 in room 6 Temperature was 23.5 ˚C (4.3˚C degrees above target). There were no reliable facilities in place to increase humidity, which was lower than target values for the duration of the experiments

Day	Temperature ˚C		Relative Humidity %	
	Target	Mean Recorded & St. Dev. (All Rooms)	Target	Mean Recorded & St. Dev. All rooms
0	32.0	30.1 1.6	43%	15.9 % 5.7
1	32.0	29.5 1.2	45%	16.5 % 5.7
2	32.0	29.3 0.7	48%	18.3 % 6.0
3	31.5	28.8 0.3	49%	20.9 % 6.7
4	31.0	28.7 0.3	50%	25.4 % 7.0
5	30.5	28.8 0.9	55%	29.8 % 2.9
6	30.0	28.3 0.4	59%	25.6 % 3.4
7	29.5	28.2 0.6	57%	27.3 % 4.6
8	29.3	27.6 0.4	60%	27.2 % 8.6
9	28.8	27.6 0.9	62%	25.0 % 6.0
10	28.5	26.5 0.8	62%	28.0 % 4.8
11	28.0	27.2 0.7	72%	31.7 % 2.7
12	27.5	27.5 0.7	70%	28.3 % 10.2
13	26.8	26.2 0.4	70%	34.6 % 9.5
14	26.5	25.9 0.5	67%	33.4 % 7.1
15	26.4	26.4 0.9	68%	29.3 % 8.5
16	25.8	25.2 0.5	67%	28.4 % 5.0
17	25.5	24.8 0.5	73%	32.2 % 6.5
18	24.8	24.7 0.7	74%	32.0 % 5.6
19	24.5	24.2 0.5	73%	31.0 % 8.9
20	23.8	23.6 0.7	67%	30.0 % 7.5
21	23.5	22.8 0.5	76%	33.5 % 7.1
22	22.0	22.5 0.7	69%	40.8 % 13.2
23	21.8	23.2 1.6	69%	43.5 % 11.3
24	21.4	21.9 1.0	72%	43.9 % 5.7
25	21.0	21.9 1.1	72%	41.2 % 6.7
26	20.8	21.7 1.8	71%	41.2 % 7.6
27	20.5	21.7 1.5	75%	36.0 % 10.1
28	20.0	21.5 1.7	74%	39.3 % 10.8
29	19.5	21.2 2.1	77%	42.3 % 9.8
30	19.2	20.9 2.0	76%	41.1 % 8.3
31	18.8	19.7 0.9	76%	39.3 % 5.7
32	18.4	19.7 1.1	75%	38.3 % 8.9
33	18.0	19.5 0.8	77%	45.3 % 8.7
34	18.0	20.1 0.6	71%	43.2 % 13.0
35	18.0	19.9 1.1	74%	43.0 % 10.9
36	18.0	19.3 0.8	71%	48.4 % 12.5
37	18.0	18.8 0.5	79%	47.3 % 11.1
38	18.0	18.4 0.6	78%	44.3 % 12.5
39	18.0	18.3 0.6	74%	44.3 % 15.0
40	18.0	18.4 0.7	76%	48.8 % 9.7
41	18.0	19.2 0.6	76%	52.7 % 10.2
42	18.0	19.7 0.7	76%	47.7 % 9.3
43	18.0	18.8 0.3	76%	46.4 % 10.7
44	18.0	18.8 0.5	76%	44.5 % 7.1

VI. II) LIGHTING CONDITIONS

There were three treatments in the current experiment; (A) UVA wavelengths but no UVB, (B) including both UVA and UVB wavelengths and the control (C) with no UV wavelengths, representative of commercial practice. Each treatment was replicated across 2 rooms (figure VI.II.a). The main light source used in all rooms for this experiment was the Agricultural Lighting Induction System (ALIS) which consisted of 4 x 8 watt clip-on LEDs provided by Greengage Lighting Ltd (Edinburgh, UK), installed 170cm from the ground.

All rooms were also fitted with a single 18watt 12% UVB D3+ T8 florescent light (Arcadia Products plc, Surrey, UK), in a reflector, powered by a high frequency 18 watt electronic ballast (Komodo, Leicestershire, UK). The fluorescent lights consisted of two end caps, each connected to the main ballast by 150 cm of cable, that attached to either end of the fluorescent tube. The fluorescent lamp, fitted in the reflector, provided the UVA and UVB wavelengths for treatment B, and was suspended from a length of steel cable, secured using cable-ties, at a height of 50cm from the ground to provide 30 µW/cm² of UVB at chick head height when measured with a UV meter (Solarmeter® Model 6.2, Pennsylvania, USA). The height of the fluorescent lamp was altered by attaching further cable ties to shorten the length of wires suspending the lamp as the chickens grew, and the corresponding lamp height was replicated across the other treatments (figure VI.II.b). It was necessary to fit these fluorescent lights in all rooms as they create a localised patch of higher light intensity with a spectral output distinct from the ALIS LEDs. The higher light intensity of the fluorescent light also masks the differences between the UVA and control treatments as measured by the spectrometer when the fluorescent light is switched on (as seen in figure VI.II.c.1- VI.II.c.4). However, in treatments A and C the fluorescent lights were fitted with clear CON-TROL-CURE® UV Blocking films (Epak Electronics, Somerset, UK). A single clip on UVA LED (Greengage Lighting Ltd, Edinburgh, UK) was added to the ALIS in treatment A to provide UVA wavelengths (figure VI.II.c).
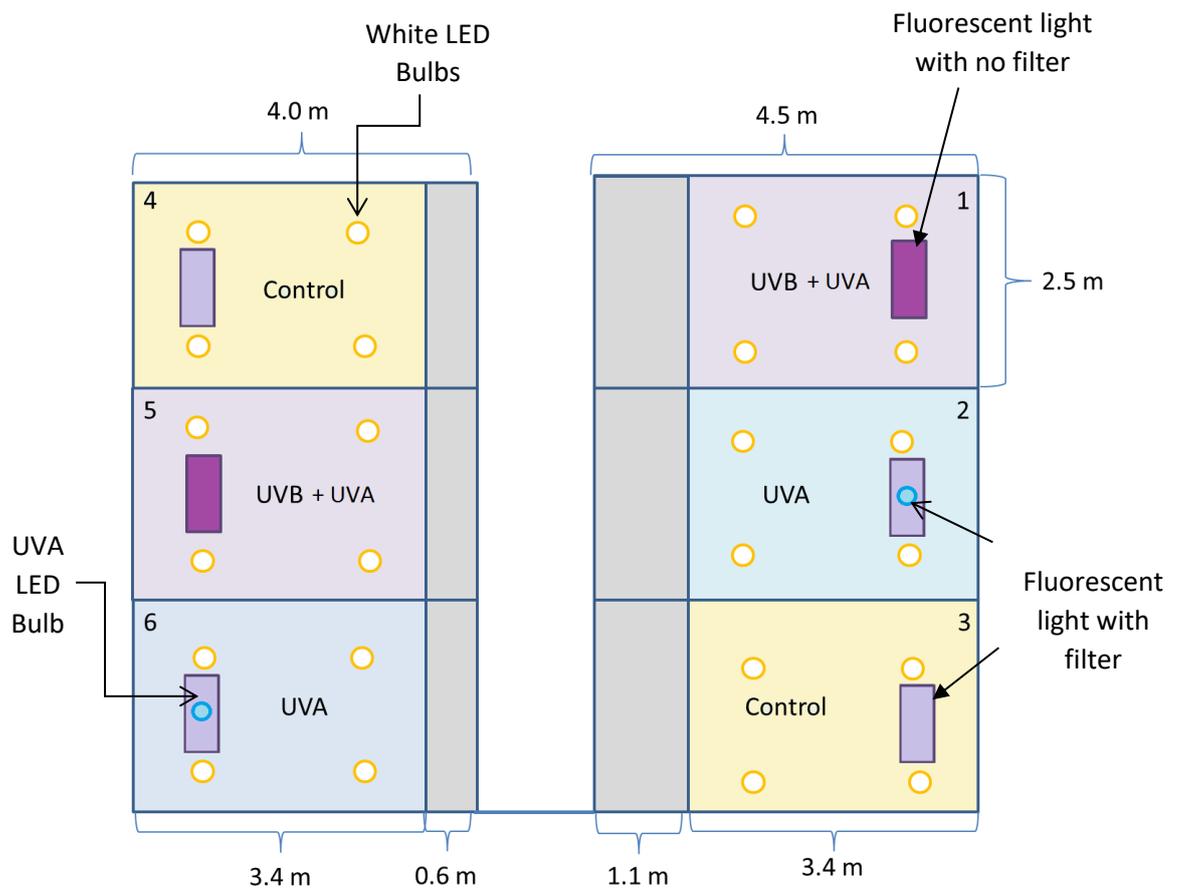
Figure VI.II.a- Dimensions and equipment layout of the six trial rooms.
Each treatment is fitted with 4 white LED lights and a UV fluorescent light fitted in a reflector. The UVA and UVB wavelengths in treatment B (room 1 and 5) are produced by the fluorescent light. UV wavelengths from these lights are blocked by a filter for the control room (rooms, 3 + 4) and treatment A (room 2 + 6). An additional UVA LED provides UVA wavelengths in treatment A.

Figure VI.II.b - The fluorescent light and reflector

which was installed in every Room. The light provided the UVA and UVB wavelengths in treatment B, and was suspended at a height of 50cm to provide 30µw/cm^2 UVB at chick head height. Lamp height was adjusted as chicks grew and replicated across other treatments. In the control treatment (no UV) and treatment A (UVA only) the fluorescent lamp was fitted with clear plastic filters to block UV wavelengths. Fluorescent lights were on for 8 hours of the total 18 hour photoperiod in all treatments.
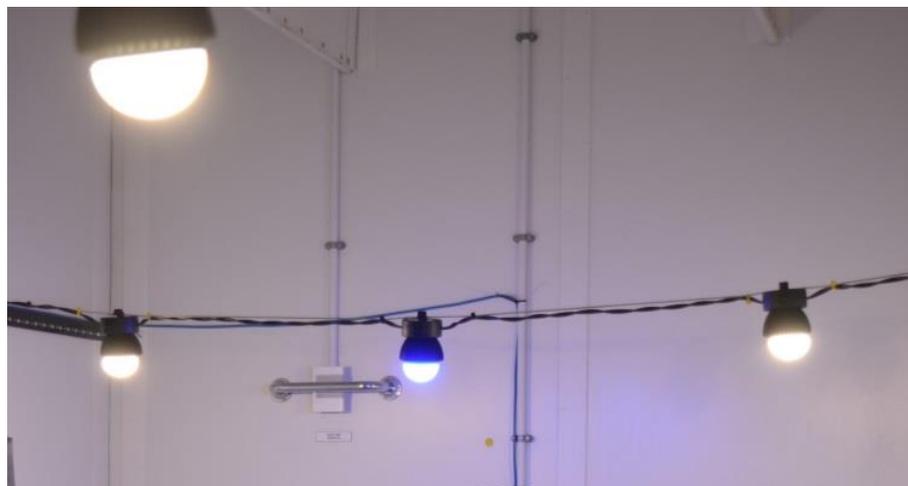
Figure VI.II.c- The Agricultural Lighting Induction System (ALIS)

showing the 8-watt clip on LED bulbs used in all pens. The UVA LED shown in this image was used in room 2 and 6, for treatment A, providing UVA wavelengths only.

Prior to the introduction of the birds the light conditions of all rooms were measured with a spectroradiometer (Model, FieldSpec® HandHeld 2 with a wavelength range of 325-1075nm and an accuracy ±1 nm, ASD inc. Colorado, USA). Spectrometry readings were taken along the midline of each room at 1, 2 and 3 meters away from the back wall at a height of 25cm from the ground. This was to ensure light intensity was approximately the same across all conditions. Raw data were extracted using ViewSpec™ Pro software (ASD inc. Colorado, USA) and light intensity was calculated in "clux" as described by by Nuboer *et al,.* (1992b) and Prescott and Wathes (1999) to ensure light intensity was approximately the same (when adjusted to the spectral sensitivity of the chicken) across all conditions using equation V.V

The mean clux measurements for all rooms obtained 1, 2 and 3 m from the back wall when the fluorescent lights were switched on were: 178.4 SEM 10.7, 19.0 SEM 0.8 and 19.0 SEM 0.5. The wavelength composition of each lighting treatment as a percentage of the total clux value is shown in table VI.II.a. The Irradiance (W/m^2) of wavelengths (nm) in each treatment measured at 1m intervals along the midline of the room at bird height is also shown in figures (VI.II.d.1-VI.II.d.4).

The same photoperiod was maintained across all lighting treatments. The ALIS system was controlled by an automated DTD (Dusk till Dawn) Lighting Processor Control, (Greengage Lighting Ltd), which incorporates 30 minutes of "dawn" and "dusk" dimming at either end of the programmed photoperiod. The scotoperiod was programmed to start at 11pm as single hour of darkness on the day the chicks arrived, increasing by an hour each night, until 6 hours of consecutive darkness was achieved (11pm-5am). The fluorescent lights were controlled by separate mechanical timers (Maplin, Rotherham, UK) programmed to switch on between 5:30-9:30am and 4:30-8:30pm for a total of 8 hours of the 18-hour photoperiod.

Table VI.II.a- mean illuminance (clux) and spectral composition of lighting treatments

Clux values and for each room were measured at meter intervals across the midline of the rooms. Clux was calculated from spectroradiometry data using the equation I = (w.s.683)/(12.566. d²) as described by Nuboer et al (1992b) and Prescott and Wathes (1999). In the current experiment, it is appropriate to remove distance from the equation as the illuminance was measured directly at bird level. Mean clux values for each treatment are presented with the SEM. The wavelength composition of each treatment as a percentage of the total clux value is also presented with SEM.

nm	1m (under the flourocent light when switched on)						2m						3m					
	control		UVA		UVA+UVB		control		UVA		UVA+UVB		Control		UVA		UVA+UVB	
Mean Total Illuminance (clux) of lighting conditions	181.20	19.07	170.89	32.42	183.23	14.14	19.75	< 0.01	20.77	0.56	16.50	0.53	19.73	0.29	19.63	0.22	19.85	< 0.01
wavelength composition of lighting conditions, shown as a mean % total clux illuminance and Standard error for the two treament rooms.																		
% 355-415 nm	1.01	0.01	1.11	0.02	2.54	0.02	0.06	0.06	1.11	0.25	< 0.01	< 0.01	0.01	0.01	0.58	0.11	0.01	< 0.01
% 416-480 nm	22.62	0.11	23.02	0.20	23.11	0.03	11.62	1.25	15.87	0.95	11.34	0.17	11.31	0.89	13.45	0.42	11.30	0.09
% 481-550 nm	39.17	0.12	39.42	0.02	38.67	0.18	27.31	0.45	26.75	0.46	26.99	0.28	26.80	0.21	26.67	0.10	26.88	0.29
% 551-650 nm	34.29	0.21	33.83	0.25	33.08	0.17	56.80	1.76	52.48	0.28	57.58	0.04	57.68	1.14	55.30	0.27	57.73	0.14
% 651+ nm	2.91	0.23	2.62	0.00	2.58	0.01	4.21	0.00	3.79	0.04	4.09	0.07	4.21	0.02	3.98	0.05	4.07	0.05
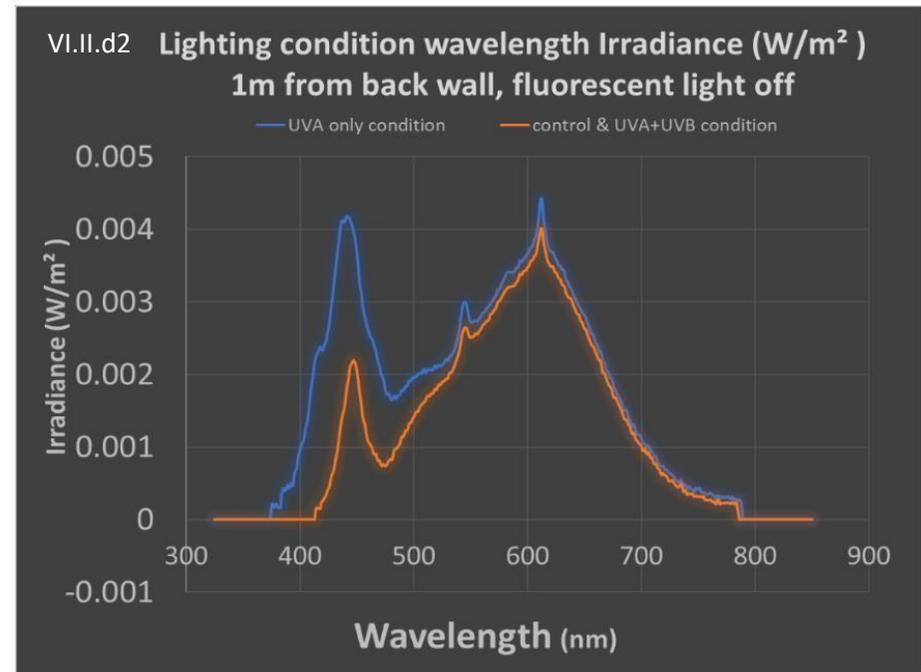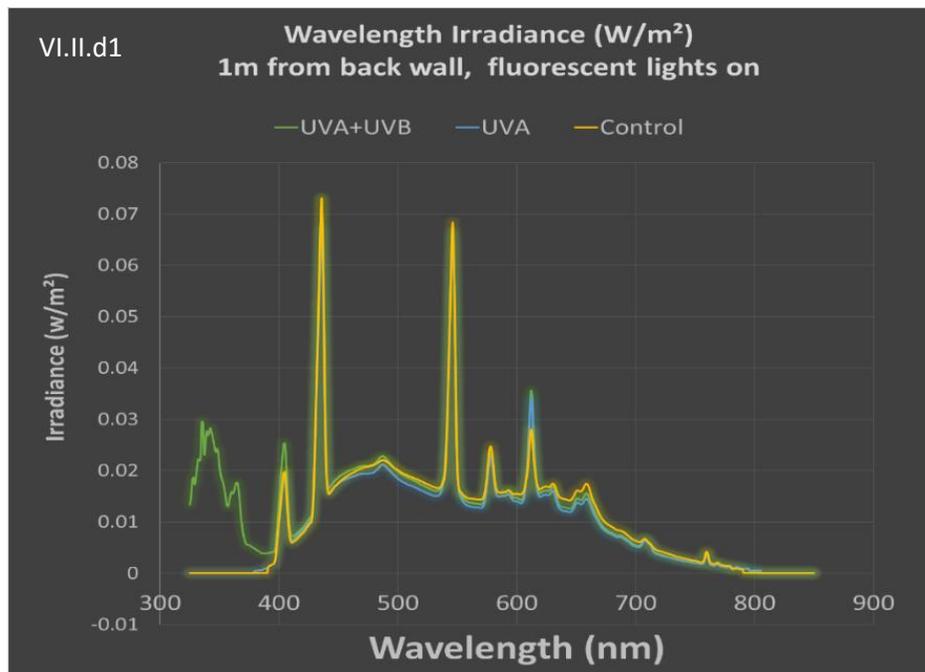
Figures VI.II.d1 and VI.II.d2 - Mean spectroradiometry measurements

25cm from the ground 1 meter from the back wall (directly under the fluorescent light). Figure VI.II.d1 shows data when the fluorescent lights are switched on (for 8 hours of the total 18-hour photoperiod). These lamps provide UVA and UVB wavelengths in treatment B, but are blocked by a clear filter in treatment C (control) and treatment A (UVA only). The UVA wavelengths in treatment A are provided by a UVA LED which also increases the amount of violet and blue visible light compared to the spectrometry profile of the white LEDs alone. This is clear in figure VI.II.d2, which shows the spectrometry readings 1m from the back wall when the fluorescent lights are off meaning treatments B and C have the same spectral composition.
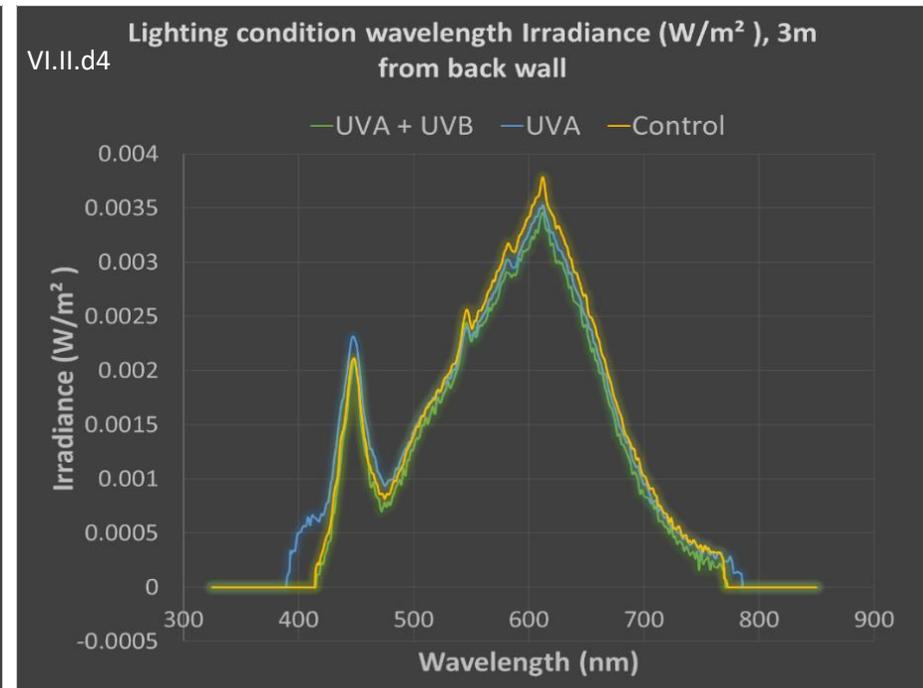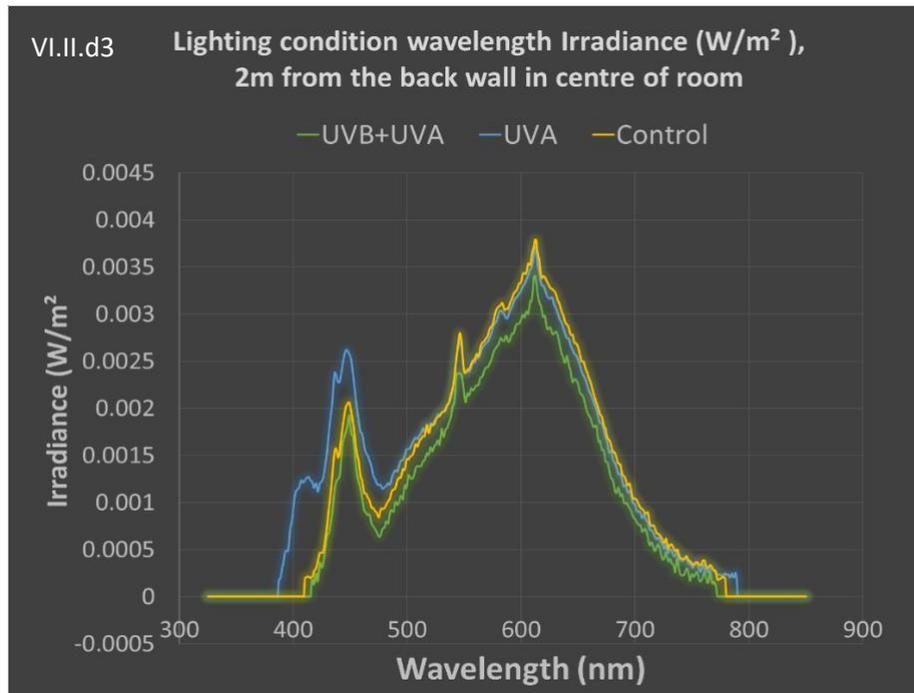
Figures VI.II.d3 and VI.II.d4 - Mean spectroradiometry measurements

for the lighting conditions taken two (c3) or three (c4) meters from the back wall 25cm from the ground when the fluorescent lights are on. The figures illustrate how the UVA provided in treatment A by the UVA LED extended across the whole room, whereas the UVA and UVB provided in treatment B by the fluorescent light is much more localised and undetectable a meter away from the fluorescent lamp. Thus, broiler chickens could self-select their exposure to UV in treatment

The fluorescent lights were not left on for the whole photoperiod to reduce the risk of overexposure of UVB, as overexposure to these wavelengths can induce neoplasia (Moan, et al., 2013) and ocular damage (Yam & Kwok, 2014). There are currently no guidelines available for the use of UVB for broiler chickens. Therefore, the level of exposure selected in the current study was very low and based on the recommendations for non-avian reptiles (Baines, 2016) and previous studies on domestic chickens (Fleming, 2008; Edwards, 2003). However, the other implication of this was that UVA was provided for the whole photoperiod (18 hours) in treatment A (UVA LED), but only 8 hours in treatment B (fluorescent light with no filter).

As the exposure times and dispersion of the UVA and UVB wavelengths within treatment A and treatment B differed these two treatments are not directly comparable to each other but are both comparable to the control treatment. Based on spectrometer readings UVA was detectable across the whole pen in treatment A. However, based on spectrometry data, the UVA and UVB in treatment B covered a floor space of approximately $1m^2$, so a reduced percentage of birds able to obtain UV exposure in treatment B (table VI.II.b) The implications of this will be discussed.

Table VI.II.b- Estimated maximum percentage UV exposure in treatment B
Estimated maximum percentage exposure is calculated based on a UV exposure area of $1m^2$, expected as hatched performance at 0-42 days old (Aviagen, 2012), the maximum target stocking density of $33kg/m^2$ and the total number of birds kept in the room (which decreases throughout the study when birds are culled to assess post-mortem measures)

age (days)	expected weight (g)	birds per room able to obtain UV	maximum percentage use estimate
0	42	107	100%
10	217	107	100%
20	844	39	39%
30	1658	20	21%
42	3046	12	16%

VI. III) GENERAL DATA COLLECTION

To monitor growth, all broilers were weighed individually using an electronic weighing balance (Sartorius IP65, Goettingen, Germany). Each animal was identified by their wing tag number and placed into a large bucket on the scales for their weight to be individually recorded. The scales were levelled after being moved into each room. Flock one (rooms 1,2 and 3) were weighed at 8, 15, 22, 27 and 34 days old. Flock 2 (rooms 4, 5 and 6) were weighed at 8, 15, 20 and 27 days old.

A sample of 6 birds per room (12 birds per condition) was culled at ages 9, 21 and 30 to assess the birds' development. Birds were selected by researchers by catching animals from six different areas of the pen. The room was visualised as six equal sections and the order a bird was caught from each section was determined using a random number generator and repeated until six birds had been captured. On days when dissections were performed the room order was also randomised. Birds were referred to by the experimenter based on their room numbers and wing tag numbers rather than by their treatment exposure. However, it was not possible to ensure all researchers and staff remained blinded to which treatments had been allocated to which rooms when collecting all data.

Final depletion took place over 5 days when birds were 35 (rooms 1, 2, 3 only) 41 (rooms 4,5, 6 only) 42, 43, 44 and 45 days old. While efforts were made to collect data from the two flocks at the same ages, this was not always possible due to the availability of technicians and the time required to collect data from large numbers of animals.

All birds were euthanised using an overdose of Pentobarbital Sodium via the intraperitoneal route for 9-day old broilers and by intravenous wing vein injection for older birds. Final body weight was obtained after confirmation of death by cervical dislocation. All birds were sexed, (n.Females = 293, n.Males = 287) and the left and right legs were dissected at the hip and

weighed along with the left and right pectoralis major (p. major) and pectoralis minor (p. minor) (n = 142 per condition). The left and right eyes of n = 381 broilers were enucleated and weighed. Mortalities and culls for health reasons (n = 50) were recorded as part of daily husbandry checks. Birds were culled for health reasons if they were suffering from torticollis that restricted their ability to eat and drink, or if they had a gait score above three (further detailed in section 2.2.c). Mortalities were recorded for each room during husbandry checks performed by animal care staff (checks were undertaken at least twice a day). The birds wing tag number was recorded before the body was immediately removed for biosecurity reasons. Necropsies and sexing of these birds were not performed.

CHAPTER ONE

1. THE EFFECT OF SUPPLEMENTARY ULTRAVIOLET WAVELENGTHS ON PERFORMANCE INDICATORS

1.1) INTRODUCTION

The domestic chicken possesses tetrachromatic colour vision and the wavelengths of light they are exposed to have been shown to influence their behaviours (Kristensen et al., 2007) and production traits (Lewis and Morris, 2000).

There is growing interest in the applications of ultraviolet wavelengths within the poultry industry. Exposure to UVB wavelengths, within 280–315 nm, facilitates endogenous synthesis of vitamin D and has been associated with improved growth, increased body weight, and reduced incidence of tibial dyschondroplasia and rickets in male broilers (Edwards, 2003). UVB exposure also improved bone mineral density, egg production, and levels of vitamin D in the egg yolk of caged laying hens (Wei et al., 2020). Dietary supplementation of vitamin D metabolites cholecalciferol and hydroxycholecalciferol were shown to improve feed efficiency, increase final body weights, and increase breast meat yield in broiler chickens under a range of conditions (Yarger et al., 1995; Fritts and Waldroup, 2003; Fritts and Waldroup, 2005). Evidence suggests Vitamin D improves breast meat yield through the stimulation of satellite cell activity (Hutton et al., 2014) and increased protein synthesis (Vignale et al., 2015). Therefore, UVB provision may be a promising strategy for improving the growth of broilers while supporting their rapid skeletal development and reducing leg weakness, which is a key welfare and economic concern.

UVA wavelengths are visible to chickens (Prescott and Wathes, 1999; Osorio et al., 1999), yet are typically absent from indoor poultry housing, though artificial UVA lighting has been shown to positively influence activity levels and the performance of comfort behaviours (Kristensen *et al.,* 2007), exploratory behaviours (Maddocks *et al.,* 2001) and lower the fear responses of broilers (James et al., 2018; House, et al., 2020) and laying hens (Sobotik, et al., 2020). However, little research has been conducted to investigate the effects of UVA on the growth and performance of broiler chickens.

Most studies comparing the effects of short and long wavelengths of light on the weight gain of broilers (between the ages of 4-11 weeks) indicate a trend towards improved (Foss, *et al.,* 1967; Johnson, *et al.*, 1982; Prayitno, *et al.,* 1997a; Prayitno, *et al.,* 1997b), or significantly better, (Foss, *et al.,* 1972; Wabeck & Skoglund, 1974; Rozenboim, *et al.,* 1999) growth of birds under violet-green light (415-560 nm) when compared with growth under red (> 635 nm) or white light. However, light sources with different spectral compositions often cannot be directly compared and many studies (Foss, *et al.,* 1967; Foss, *et al.,* 1972; Johnson, *et al.*, 1982; ; Rozenboim, *et al.,* 1999; Wabeck & Skoglund, 1974) have inadvertently confounded the influences of wavelength composition with the influence of light intensity (Lewis and Morris, 2000). A Review of these studies by Lewis and Morris (2000) noted the growth of broiler chickens under white and red light was similar, suggesting long wavelengths may suppress growth.

Overall, a decrease of 50g body weight for each 100nm increase in wavelength between 530-750nm was observed in broilers (Lewis & Morris, 2006), which was thought to be due to the higher photo-stimulatory effects of longer wavelengths, which can more easily penetrate the cranial tissues of birds and stimulate the hypothalamic photoreceptors, inducing an earlier rate of sexual maturity and thus heightened activity and aggression (Lewis and Morris, 2000).

As lighting technology continues to advance, it is important to understand the impacts of wavelength composition on broiler performance. LEDs are gaining popularity in industry as they are energy efficient, eliminate exposure to mercury found in fluorescent bulbs, and offer opportunities to tailor the wavelength composition of the light environment more precisely (Yeh & Chung, 2009; Pimputkar, et al., 2009).

Broiler chickens reared under LED light sources were found to have reduced fear and stress responses (Huth and Archer, 2015), improved tibia breaking strength (Akşit et al., 2017), and more favourable feed conversion ratios compared with broilers reared under compact fluorescent lights (Huth and Archer, 2015; Mendes et al., 2013) or standard fluorescent lights (Akşit et al., 2017).

Additionally, the distinct spectral outputs of LED have provided a way to study the impacts of monochromatic and mixed spectra light environments on broiler chicken performance more precisely.

Green light was found to promote the growth of broiler chickens during early development, whereas blue light enhanced growth during the later stages of development (Rozenboim *et al.,* 2004; Cao *et al.,* 2008; Cao *et al.,* 2012). Evidence suggests green and blue light promote the growth of broiler chickens by increasing satellite cell proliferation (Halevy et al., 1998) and stimulating testosterone secretion (Rozenboim *et al.,* 2004; Cao *et al.,* 2008). Monochromatic green and blue light was also found to improve the meat quality of broilers compared to those reared under incandescent lighting (Karakaya, et al., 2009).

Further benefits of blue and green wavelengths in broiler chickens include enhanced cellular and humoral immune responses as measured through T-lymphocyte proliferation assays and antibody responses to a Newcastle disease vaccine (Xie et al., 2008). which is thought to be facilitated by alleviation of the stress responses through a reduction of circulating interleukin-1β (Xie et al.,

2008). Compared with monochromatic conditions, mixed green and blue wavelengths may further improve broiler performance (Yang et al., 2016).

Wavelength composition generally does not impact broiler mortality rates (Wabeck and Skoglund, 1974; Lewis and Morris 2000; Rozenboim et al., 2004; Cao *et al.,* 2008; Cao *et al.,* 2012; Mendes *et al.,* 2013; Rogers *et al.,* 2015), though green light was associated with higher mortality in female broilers aged under 7 weeks (Proudfoot and Sefton, 1978) and lower mortality in broiler breeders between 10 and 40 wk old (Cave, 1990) compared with those reared under white light. However, more studies investigating the impacts of wavelengths do not report mortality (Archer, 2015; Karakaya, *et al.,* 2009; (Mohamed, et al., 2017; Mohamed, *et al.,* 2020; Sobotik, *et al.,* 2020; Xie *et al.,* 2008;Yang *et al.,* 2016), or state that it was measured without reporting numbers or wheter there were any differences bettween treatments (Akşit *et al.,* 2017; Archer, 2017; House *et al.,* 2016; Huth & Archer, 2015), which makes it difficult to draw conclusions.

Additionally, light spectra influence the regulation of melatonin production and other endocrine biorhythms in chickens (Apeldoorn, *et al*., 1999; Gwinner, *et al*., 1997), which impact feed consumption, energy metabolism, heat production and growth hormone concentration with clear implications for poultry production (Calislar, *et al.,* 2018; Zeman, *et al.,* 1999). Different wavelengths have been shown to alter the expression of circadian clock genes in both the pineal gland (Jiang, *et al.,* 2016) and the retina (Bian, *et al*., 2020; Cao, *et al.,* 2017). Shorter wavelengths are more efficient at influencing pineal activity than longer wavelengths in birds (Csernus, *et al.,* 1999), via the photoreceptive molecule pinopsin (Okano, *et al.,* 1994). Illumination with UVA light dramatically decreases the action of AANAT and melatonin systhesis in the pineal gland and the retina via N-methyl-D-aspartate (NMDA) glutamate receptors (Rosiak & Zawilska, 2005). Therefore, it is possible that UVA could illicit similar effects observed under other monochromatic short wavelength conditions composed of blue or green wavelengths.

The aim of the current study was to evaluate the impact of supplementary UVA only (provided by a LED light source) or a combination of UVA and UVB wavelengths (Provided by a fluorescent light source) on performance indicators in broilers chickens kept at commercial stocking densities and fed on a commercial diet. The Provision of UVB wavelengths, to enable endogenous vitamin D synthesis, may improve the growth and breast yield of broiler chickens. If the addition of UVA wavelengths (in conjunction with a small increase of the amounts of visible blue light) effects broilers in similar ways observed under monochromatic short wavelength conditions, then similar improvements in growth may be observed (table 1.1).

The use of UVA LEDs is a novel approach, which has not been implemented in previous studies and could potentially be implemented on a commercial scale. UVA is thought to benefit broiler welfare through increasing activity levels, decreasing fearfulness and increasing the expression of comfort behaviours. Therefore, it would be worthwhile to investigate the impacts of these wavelengths on production traits as adoption of UVA in commercial settings may be reduced if broiler performance is impaired.

Performance indicators observed in the current study were; mortality rate, weight gain, average daily weight gain, and breast and leg weights.

Table 1.1 – Summary of hypothesised impacts of UVA and UVB wavelengths,
*Based on the findings previous studies UVA and UVB may improve growth and performance. * These impacts may be observed if UVA wavelengths have similar impacts on broiler chickens to green and blue wavelengths.*

	Potential impact	
Performance Indicator	**UVA**	**UVB**
Weekly weights	increased (later growth)*	increased
average daily gain	increased (later growth)*	increased
End weight	increased	increased
mortality	unknown	unknown
Breast weight	increased*	increased
Leg weight	increased*	increased

1.2) METHODS

Details of the Animals used, general husbandry procedures, lighting equipment, and wavelength composition of the light treatments can be found along with details of live and post-mortem data collection in section VI. The following performance indicators were examined: mortality, weekly live weight, end weight, average daily gains (g) and leg and breast muscle weights.

1.2.a) Mortality

All broilers (n = 638) were assigned a binary outcome of 0 (culled at the end of the experiment) or 1 (mortality or culled for health reasons).

A generalised linear model (glm) with binomial family (analogous to a logistic regression) was constructed to investigate the impact of multiple independent variables (age, flock and lighting treatment) and interactions between them on the dependent variable of mortality in R statistical software. Sex could not be included as an independent variable as this was not recorded for all mortalities. Final models were selected using the flow charts detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether there was a significant change in model fit (chi-squared test).

1.2.b) Weekly weights

General linear models (glm) were constructed to investigate the impact of multiple independent variables (sex, flock and lighting treatment) and interactions between them on the dependent variable of Weight (g) at 8, 15, 22, 27 and 34 days old (Flock 1) or 8, 15, 20 and 27 (Flock 2) days old in R statistical software. In cases where data were collected at different ages for each flock these weights were modelled separately. Final models were selected

using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether there was a significant change in model fit (chi-squared test).

1.2.c) Average daily gain

The average daily weight gain was calculated for individual broilers between each weekly weight recording.

$$Average\ daily\ gain\ (g) = \frac{new\ weight - previous\ weight}{number\ of\ days\ between\ weighing}$$

General linear models (glm) were constructed to investigate the impact of multiple independent variables (Sex, flock and lighting treatment) and interactions between them on the dependent variable of average daily gain (g) between the ages of 8-15, 15-22, 22-27 and 27-34 days old (Flock 1) or 8-15, 15-20 and 20- 27 days old (Flock 2) in R statistical software. In cases where data were collected at different ages for each flock these weights were modelled separately. Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether there was significant change in model fit (chi-squared test).

1.2.d) End weights

General linear models (glm) were constructed to investigate the impact of multiple independent variables (sex, flock and lighting treatment) and interactions between them on the dependent variable of End Weight (g) at 9, 21, 30, 35 (rooms 1, 2, 3 only) 41 (rooms 4,5, 6 only) 42, 43, 44 and 45 days old in R statistical software. Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude

variables, based on whether there was a significant change in model fit (chi-squared test).

1.2.e) Breast and Leg weights

The average breast and leg weight was calculated for individual broilers. General linear models (glm) were constructed to investigate the impact of multiple independent variables (Sex, flock and lighting treatment) and interactions between them on the dependent variables on mean breast and leg weights (g) at 9, 21, 30, 35 (rooms 1, 2, 3 only) 41 (rooms 4,5, 6 only) 42, 43, 44 and 45 days old in R statistical software. Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether there was a significant change in model fit (chi-squared test).

The leg and breast weight as a percentage of total end body weight was calculated for individual broilers. The leg mass relative to breast mass was investigated by dividing the percentage of leg weight by the percentage of breast weight, giving a value that was >1 where leg weight exceeded breast weight and < 1 where breast weight exceeded leg weight. General linear models (glm) were constructed to investigate the impact of multiple independent variables (Sex, flock and lighting treatment) and interactions between them on the dependent variables of breast and leg weight as a percentage of body weight and leg mass relative to breast mass at 9, 21, 30, 35 (rooms 1, 2, 3 only) 41 (rooms 4,5, 6 only) 42, 43, 44 and 45 days old in R statistical software. Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether there was a significant change in model fit (chi-squared test).

Table 1.2 -Summary of main independent and dependent variables of interest
*Independent variables and interactions between them were included in generalised linear models (n = 37 models; 1 for mortality, 18 for males and 18 for females) to determine their effects on the performance indicators of interest; mortality, weekly weights and average daily gains, end weight and mean average leg and breast weight. * Age was included in models where data were collected at multiple time points (end weight, mortality, breast weight /percent, leg weight/percent, and leg weight as proportion of breast weight).*

Independent variables	Dependent variables
Sex	Mortality
Flock	**Weight (g)** at 8, 15, 21, 27, 34 (flock 1) or 8, 15, 20, 27, (flock 2) days old
Lighting treatment	**Average daily gains (g)** between 8-15, 15-21, 21-27, 27-34 (flock 1) or 8-15, 15-20, 20-27, (flock 2) days old
Age *	End weight (g)
	Average breast weight
	Average leg weight
	Percentage breast weight (of total end weight)
	Percentage leg weight (of total end weight)
	Percent leg /Percentage breast

1.2.f) Corrections for multiple testing

Corrections for multiple testing were performed for all above models using a modified Bonferroni procedure (Haccou, et al., 1992). Models were ordered by p value from lowest to highest. P values were then adjusted by multiplying all p values in each model by the total number of models (n = 37) for the first model, and then subsequently multiplying by n - 1 (for the model with the second lowest p values) followed by n- 2, then n- 3, until the p values of the model with the highest p values were multiplied by n-36 and thus stayed the same. Unless otherwise stated, corrected p values are presented within text and figures.

1.3) RESULTS

1.3.a) Mortality

Broiler chickens in the UVA only treatment had reduced mortality compared to the control treatment before corrections for multiple testing (C vs A, glm: n=638, z = -2.689, p = 0.007) (Table 1.3.a). Though after corrections for multiple testing there was no significant treatment effect (C vs A, glm: n=638, z = -2.689, p = 0.165) (Table 1.3.f). There was also a significant effect of age, with mortality less likely to occur as broilers got older (glm: n=638, z = -7.243, p < 0.001).

Table 1.3.a – Frequency of mortalities and age of occurrence
Mortality of broiler chickens in different lighting treatments and flocks. The percentage mortality for each room, treatment and for the overall trial is also shown.

Treatment	Flock (Room)	Age (Days)					Total	%
		0 - 7	8 - 14	15-25	26- 35	35+		
Control	Flock 1 (3)	0	5	1	4	6	16	15
	Flock 2 (4)	2	1	0	5	2	10	9
	Total	**2**	**6**	**1**	**9**	**8**	**26**	**12**
UVA only	Flock 1 (2)	1	0	0	0	1	2	2
	Flock 2 (6)	3	0	2	0	0	5	5
	Total	**4**	**0**	**2**	**0**	**1**	**7**	**3**
UVA & UVB	Flock 1 (1)	2	0	2	3	2	9	8
	Flock 2 (5)	3	0	0	2	3	8	8
	Total	**5**	**0**	**2**	**5**	**5**	**17**	**8**
	Overall	**11**	**6**	**5**	**14**	**14**	**50**	**8**

1.3.b) Weekly Weights

Due to significant differences in the growth curves of male and female broilers sexes were modelled separately. Log values of weights were used for statistical analysis to improve model fit.

There was no significant effect of treatment on the weights of 8-day old male broilers (table 1.3.b). However, 8-day old female broilers in the UVA treatment (glm: n=293, t = -4.993, p < 0.001) and UVA & UVB treatment (glm: n=293, t = -3.273, p = 0.025) were lighter than control females.

There was no significant effect of treatment on the weights of 15-day old female broilers. Male broilers in the UVA only treatment were lighter than control males at 15 days old (glm: n=267, t = -3.374, p < 0.022).

20-day old males (flock 2 only) in the UVA only treatment were lighter than control males (glm: n=156, t = -5.505, p < 0.001) and 20 day old females in the UVA only (glm: n=138, t = -3.068, p = 0.021) and UVA + UVB treatment (glm: n=138, t = -3.145, p = 0.017) were lighter than 20 day old control females. Males broilers at 22 days old (flock 1 only) in the UVA + UVB treatment were heavier than control males (glm: n=131, t = 4.039, p = 0.001) and there was no significant effect of treatment on the weight of 22-day old females.

Males at 27 days old in the UVA only treatment were lighter than control males (glm: n=248, t = -3.620, p = 0.007), though UVA + UVB treated males were heavier than control males (glm: n=248, t = 3.304, p = 0.021). There was no significant effect of treatment on the weight of 27-day old females.

Male broilers at 34 days old (flock 1 only) in the UVA + UVB treatment were heavier than control males (glm: n=92, t = 4.522, p < 0.001) Though there was no treatment effect on the weight of 34 day old female broilers after corrections for multiple testing (table 1.3.f).

Flock also had a significant impact on weights and was retained in all models where birds were weighed at the same age. Flock 2 males weighed significantly less than flock 1 males at 8 (glm: n=286, t = -9.313, p < 0.001), 15 (glm: n=267,

t = -8.406, p < 0.001), and 27 (glm: n=248, t = -5.461, p < 0.001) days old. Flock 2 females weighed significantly less than flock 1 females at 8 (glm: n=293, t = -6.222, p < 0.001), 15 (glm: n=276, z = -8.167, p < 0.001), and 27 (glm: n=259, z = -3.0571 p = 0.017) days old.

Table 1.3.b) - Mean weekly weights of broiler chickens

Mean weekly weights (g) and standard deviation of male and female broiler chickens in each lighting treatment. Paired characters indicate significantly different weights for broilers of the same age. Superscript numbers denote where flock weights were recorded at different ages and analysed independently.

Age (Days)	Control						
	n	Females	SD		n	Males	SD	
8	55	248	20.1	a	128	233	28.8	
15	50	591	41.6		122	602	56.0	b
20[2]	6	997	48.8	ef	88	984	84.4	d
22[1]	49	1124	107.0		41	1221	127.1	g
27	46	1567	144.3		113	1718	167.6	hi
34[1]	41	2137	183.5		31	2517	175.2	j

	UVA only						
	n	Females	SD		n	Males	SD	
8	179	223	22.6	a	28	227	31.3	
15	171	561	66.8		23	565	75.1	b
20[2]	81	865	79.0	e	21	874	84.5	d
22[1]	98	1097	102.1		7	1295	162.6	
27	161	1532	152.6		22	1582	175.6	h
34[1]	84	2251	261.1		2	2660	363.5	

	UVA + UVB						
	n	Females	SD		n	Males	SD	
8	59	218	26.2		130	244	22.0	
15	55	539	71.3		122	634	74.3	
20[2]	51	842	134.5	f	47	1000	74.7	
22[1]	8	1203	157.6		83	1318	108.0	g
27	52	1497	160.9		113	1833	159.8	i
34[1]	6	2406	409.2		59	2739	241.8	j

1.3.c) Average daily weight gain

Due to significant differences in the growth curves of male and female broilers sexes were modelled separately. Log values of weights were used for statistical analysis to improve model fit.

Between the ages of 8 and 15 days old male broilers in the UVA only treatment gained less weight than control males (glm: df=265, t = -3.759, p < 0.004) (figure 1.3.c), though males in the UVA + UVB treatment gained more weight than control males (glm: df=265, t = 3.382, p = 0.017). There were no treatment effects on female broilers between the ages of 8 and 15 days after corrections for multiple testing (table 1.3.f).

Between the ages of 15- 20 days old male broilers (Flock 2) in the UVA only treatment gained less weight than control males (glm: n=145, t = -4.868 p < 0.001). Females between 15- 20 days old also gained less weight in the UVA only (glm: df=128, t = -3.713, p = 0.003) and UVA + UVB treatments (glm: df=128, t = -3.980, p = 0.001) compared to control females.

Between the ages of 15- 22 days old male broilers (Flock 1) in the UVA + UVB treatment gained more weight than control males (glm: df=108, t = 4.160 p < 0.001). There was a trend for females between 15- 22 days old to gain less weight in the UVA only treatment (glm: df=137, t = -1.677, p < 0.096) compared to control females.

Between the ages of 20 - 27 days (Flock 2) males in the UVA + UVB treatment gained more weight than control males (glm: df= 137, t = 3.899 p = 0.002). Treatment had no significant effect on female broilers between the age of 20- 27 days.

Between the ages of 22-27 days (Flock 1) There was no significant effect of treatment on male or female broilers.

Between the ages of 27-34 days (Flock 1) male broilers in the UVA + UVB treatment gained significantly more weight than control males (glm: df= 91, t =

7.552 p < 0.001) and females in the UVA only treatment gained more weight than female control broilers (glm: df= 130, t = 5.408 p < 0.001).

There were also significant differences between flock 1 and flock 2 between 8-15 days old, with flock 2 males (glm: n=267, t = -5.968, p < 0.001) and females (glm: n=276, t = -8.098, p < 0.001) gaining less weight than flock 1.
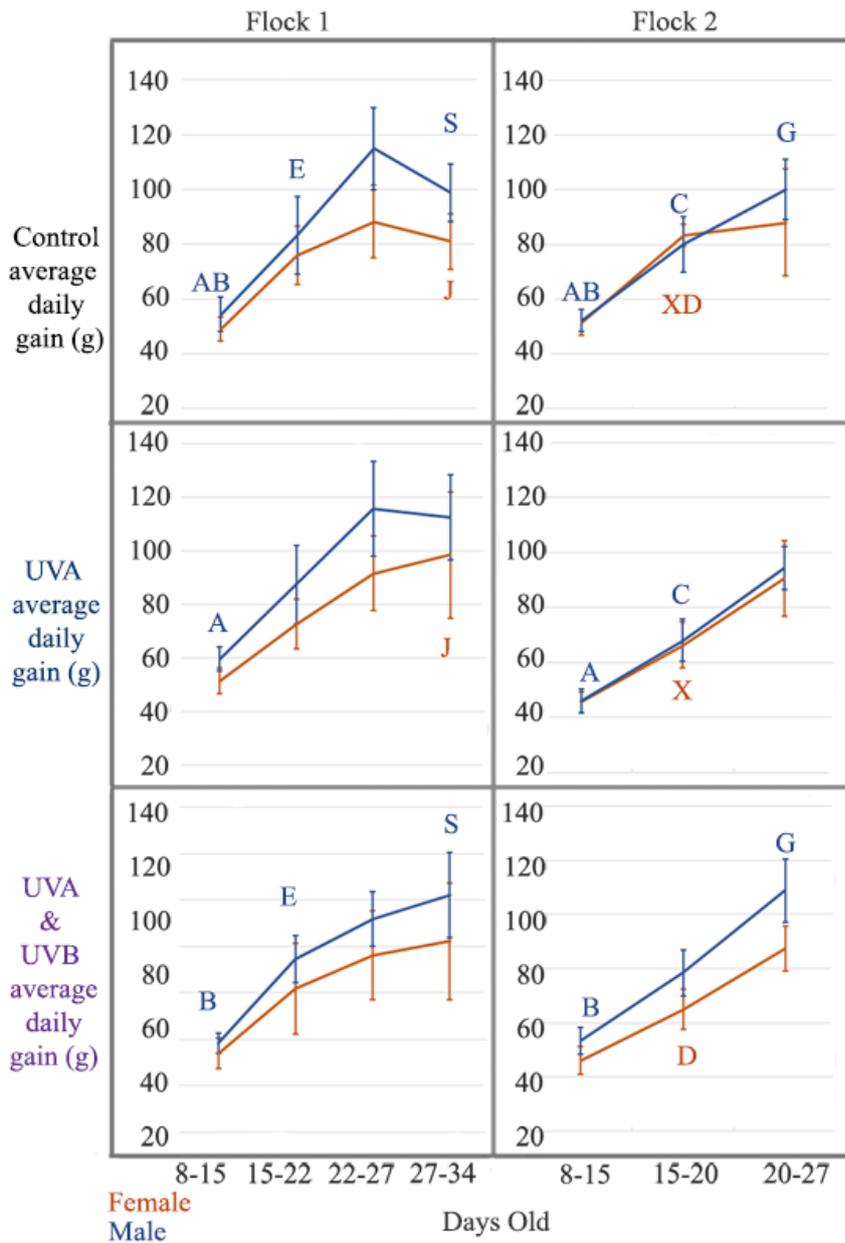
Figure 1.3.c) – Mean average daily gains for male and female broilers with and without UV supplementation.
Matching characters denote significant differences in average daily gain for the measured time period.

1.3.d) End Weights

Due to significant differences in the growth curves of male and female broilers sexes were modelled separately. Log values of all weights were used for statistical analysis to improve model fit.

End weight (Table 1.3.d) significantly increased with age for males (glm: df= 267, t = 37.731, p < 0.001) and females (glm: df= 275, t = 45.649, p < 0.001). Males in flock 2 were significantly lighter than flock 1 males (glm:df = 267, t = -4.379, p < 0.001).

Before corrections for multiple testing, male broilers in the UVA + UVB treatment had heavier end weights overall than control broilers (glm: df= 267, t = 2.451, p = 0.015), and there was a non-significant trend for females in the UVA treatment to have lighter end weights than control females (glm: df= 275, t = -1.816, p = 0.071). There were no significant treatment effects on end weight for male or female broiler chickens after correction for multiple testing (Table 1.3.f).

Table 1.3.d – Mean end weights and standard deviation for male and female broilers with and without UV supplementation

Superscript numbers denote where flock weights were recorded at different ages. End weight increased with age for male and female broiler chickens. There were no effects of lighting treatment on the end weight of broilers after corrections for multiple testing.

Age (Days)	Control					
	n	Females	SD	n	Males	SD
21	3	977	53.3	9	1102	103.6
30	2	1730	300.2	10	2008	322.7
35[1]	6	2275	364.0	4	2623	246.1
41[2]	3	3579	241.6	29	3399	346.0
42	6	2956	337.6	21	3503	278.7
43	6	3203	302.9	19	3722	299.0
44	17	3249	214.1	20	3922	267.5
45	4	3358	159.7	6	3969	245.4

Age (Days)	UVA only					
	n	Females	SD	n	Males	SD
21	10	935	95.6	2	1132	47.2
30	12	1720	131.7	0		
35[1]	9	2293	145.7	1	3003	
41[2]	11	2988	242.4	19	2917	250.3
42	28	2946	239.9	0		
43	35	3194	272.3	0		
44	49	3102	340.4	1	3708	
45	11	3144	209.6	1	3740	

Age (Days)	UVA + UVB					
	n	Females	SD	n	Males	SD
21	3	978	52.1	9	1195	81.5
30	3	1865	169.1	9	2160	223.9
35[1]	0			10	2887	212.3
41[2]	11	2847	286.9	20	3421	223.1
42	11	2933	175.6	16	3487	403.3
43	7	2955	295.9	32	3819	444.1
44	15	3079	358.7	20	3915	285.6
45	5	3412	399.4	6	4169	237.3

1.3.e) Breast and Leg weights

Due to significant differences in the growth curves of male and female broilers sexes were modelled separately. Log values of all weights were used for statistical analysis to improve model fit.

There was no significant effect of lighting treatment on broiler chicken breast (table 1.3.e.i) or leg weight (table 1.3.e.ii). Age was a significant factor for male (glm: df= 189, t = 49.478 p < 0.001) and female (glm: df= 189, t = 51.536 p < 0.001) breast weight in addition to male (glm: df= 189, t = 48.005 p < 0.001) and female (glm: df= 189, t = 47.481 p < 0.001) leg weight.

Breast weight as a percentage of body weight was significantly affected by age for male (glm: df= 189, t = 25.157 p < 0.001) and female (glm: df= 189, t = 24.435 p < 0.001) broilers (figure 1.3.e.i). There was a trend for leg percentage of total body weight to reduce as age increased for female broilers (glm: df= 189, t = -2.822 p = 0.090), but no effect of age on male leg percentage of body weight after corrections for multiple testing (table 1.3.f).

Leg percentage was also affected by Flock, with both males (glm: df= 189, t = -3.664, p < 0.001) and females (glm: df= 189, t = -5.416, p < 0.001) in flock 2 having reduced leg percent compared to flock 1.

Leg weight as a proportion of breast weight was significantly reduced by age for male (glm: df= 189, t = -24.798 p < 0.001) and female (glm: df= 189, t = -26.674 p < 0.001) broilers (figure 1.3.e.ii).

Table 1.3.e- Mean average breast weights for broiler chickens with and without UV supplementation

There was no significant effect of lighting treatment on broiler chicken breast weight.

Age (Days)	Control					
	n	Females	SD	n	Males	SD
9	5	19	3.0	7	17	2.2
21	3	88	11.5	9	97	13.1
30	2	172	31.0	10	193	41.3
42	6	344	58.8	16	390	53.4
43	4	368	25.7	16	416	50.6
44	6	353	25.9	11	455	35.1
Age (Days)	UVA only					
	n	Females	SD	n	Males	SD
9	6	16	2.9	6	18	3.1
21	10	80	9.7	2	98	22.6
30	12	173	17.4			
42	23	329	36.6			
43	20	357	52.1			
44	19	349	50.4			
Age (Days)	UVA + UVB					
	n	Females	SD	n	Males	SD
9	8	17	3.7	4	17	2.5
21	3	88	8.9	9	106	10.0
30	3	193	10.8	9	224	32.6
42	7	326	23.1	15	384	41.6
43	5	342	26.6	15	416	37.3
44	5	329	43.0	12	460	40.4

Table 1.3.e.ii – Mean average leg weights for broiler chickens with and without UV supplementation

There was no significant effect of lighting treatment on broiler chicken leg weight.

Age (Days)	Control					
	n	Females	SD	n	Males	SD
9	5	29	3.3	7	29	2.7
21	3	111	3.8	9	127	14.0
30	2	197	44.4	10	228	22.7
42	6	302	33.6	16	382	22.1
43	4	348	31.7	16	394	32.7
44	6	320	15.1	11	440	34.0

Age (Days)	UVA only					
	n	Females	SD	n	Males	SD
9	6	25	2.9	6	29	4.1
21	10	102	12.5	2	137	3.6
30	12	193	13.1	0		
42	23	301	23.4	0		
43	20	326	28.9	0		
44	19	320	36.9	0		

Age (Days)	UVA + UVB					
	n	Females	SD	n	Males	SD
9	8	27	3.9	4	29	3.1
21	3	110	7.9	9	138	9.5
30	3	206	24.4	9	240	28.8
42	7	307	11.8	15	385	48.2
43	5	303	16.7	15	410	37.5
44	5	325	42.9	12	435	26.8
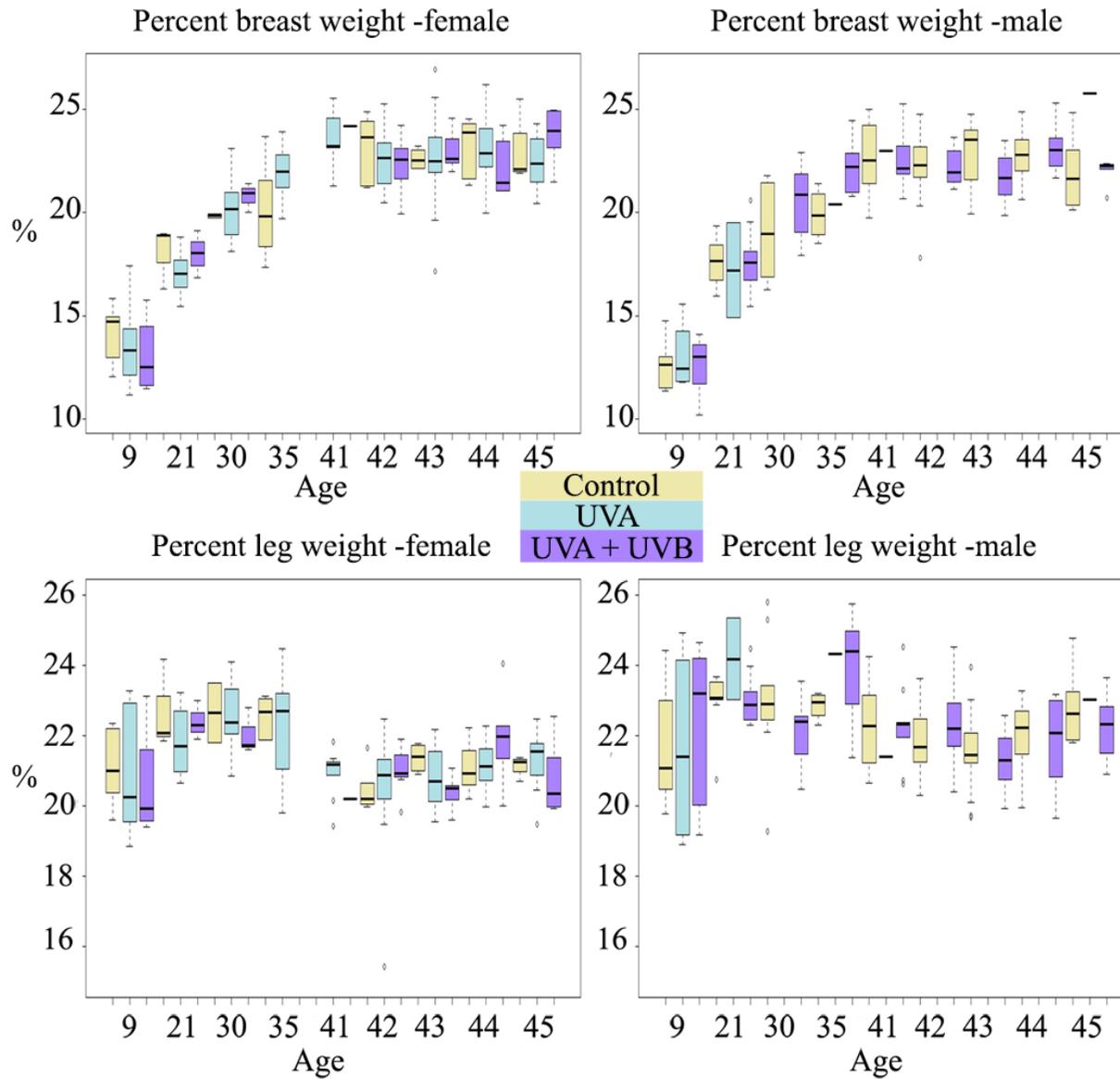
There was no impact of lighting treatment on the percentage of breast or leg weight as a percentage of body weight. Leg and breast percent increased with age for male and female broilers (p<0.001). There was a trend for increasing age to reduce leg percentage of total body weight in female broiler chickens (p = 0.090).
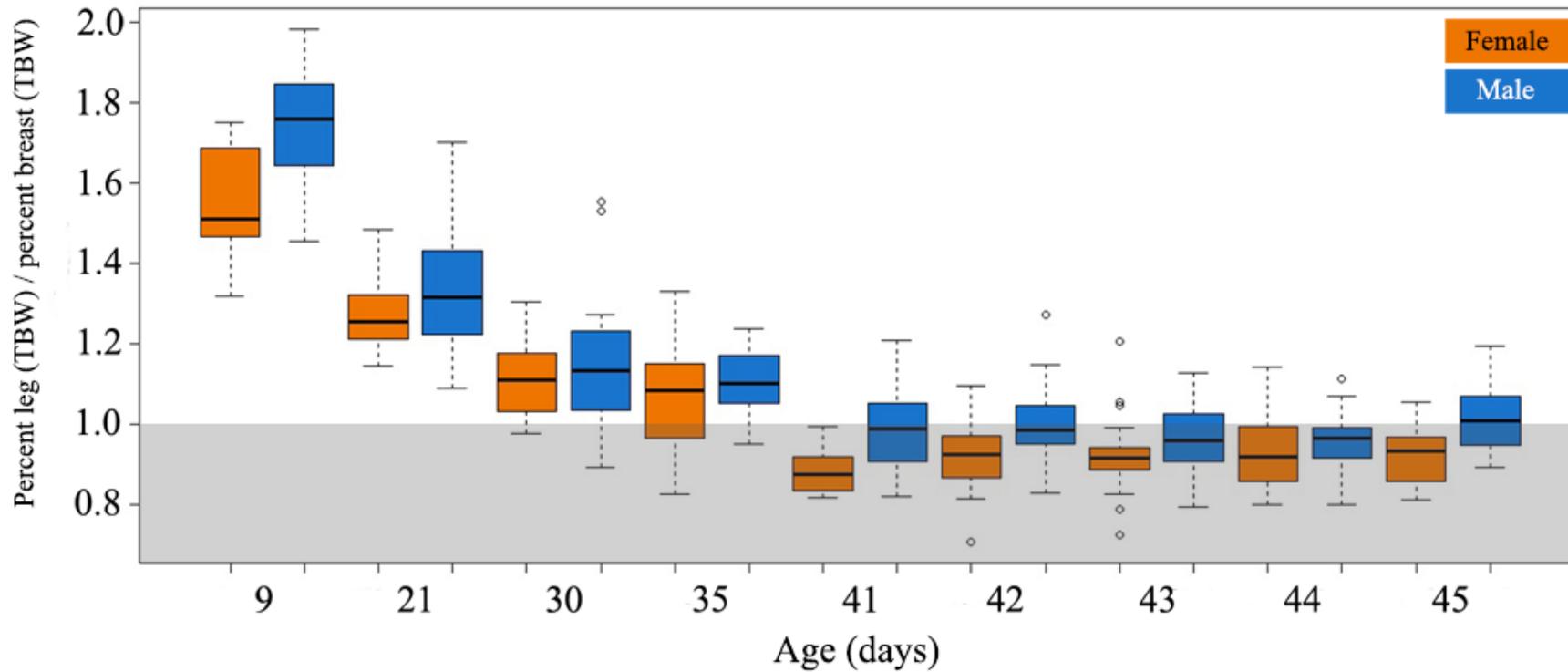
Figure 1.3.e.ii – Proportion of leg weight relative to breast weight for male and female broiler chickens between 9-45 days old

Leg weight relative to breast weight was investigated by dividing the leg percentage of total body weight (TBW) by the percentage of breast weight, giving a value that was >1 where leg weight exceeded breast weight and < 1 where breast weight exceeded leg weight as indicated by the grey portion of the figure.   Leg weight as a proportion of breast weight reduced as age increased (p < 0.001) for male and female broilers though there was no effect of UV supplementation.

Table 1.3.f – The main impacts of ultraviolet wavelength exposure on broiler chicken performance indicators

Odds ratios and 95% confidence intervals (mortality model) or estimates and SEM (other models) are shown for performance indicators of interest. Significant results (p < 0.05) from generalised linear models before corrections for multiple testing are italicised. Significant outcomes after adjustment of p values using the modified Bonferroni procedure (section 1.2.f) are emboldened

		Odds ratio	95% confidence intervals		p	Adjusted p value
	Mortality		**-**	**+**		
	Control vs UVA	0.278	0.707	0.110	*0.007*	0.165
	Age (days)	0.920	0.941	0.899	*< 0.001*	**< 0.001**
	Weekly Weights (g)	estimate	Standard error		p	Adjusted p value
(Age)	**MALES**					
8	Flock 1 vs Flock 2	-0.051	0.005		*< 0.001*	**< 0.001**
15	Flock 1 vs Flock 2	-0.040	0.005		*< 0.001*	**< 0.001**
	Control vs UVA	-0.028	0.008		*< 0.001*	**0.022**
	Control vs UVA +UVB	0.013	0.005		*0.008*	0.231
20	Control vs UVA	-0.052	0.010		*< 0.001*	**< 0.001**
22	Control vs UVA +UVB	0.034	0.009		*< 0.001*	**0.001**
27	Flock 1 vs Flock 2	-0.030	0.005		*< 0.001*	**< 0.001**
	Control vs UVA	-0.034	0.009		*0.001*	**0.007**
	Control vs UVA +UVB	0.018	0.006		*< 0.001*	**0.021**
34	Control vs UVA +UVB	0.036	0.008		*< 0.001*	**< 0.001**
(Age)	**FEMALES**					
8	Flock 1 vs Flock 2	-0.036	0.006		*< 0.001*	**< 0.001**
	Control vs UVA	-0.034	0.007		*< 0.001*	**< 0.001**
	Control vs UVA +UVB	-0.030	0.009		*0.001*	**0.025**
15	Flock 1 vs Flock 2	-0.049	0.006		*< 0.001*	**< 0.001**
20	Control vs UVA	-0.063	0.021		*0.002*	**0.021**
	Control vs UVA +UVB	-0.066	0.021		*0.002*	**0.017**
27	Flock 1 vs Flock 2	-0.019	0.006		*0.002*	**0.017**
34	Control vs UVA	0.021	0.009		*0.019*	0.112
	Control vs UVA +UVB	0.047	0.021		*0.024*	0.143
	Leg Percent (TBW)	estimate	Standard error		p	Adjusted p value
	MALES					
	Flock 1 vs Flock 2	-0.751	0.205		*< 0.001*	**0.003**
	Age	-0.016	0.009		0.081	0.732
	FEMALES					
	Flock 1 vs Flock 2	-0.991	0.183		*< 0.001*	**< 0.001**
	Age	-0.020	0.007		*0.005*	0.089.
	Control vs UVA +UVB	0.536	0.304		0.080	1.355

Table 1.3.f continued…

Average daily gain (g)		estimate	Standard error	p	Adjusted p value
(Age)	**MALES**				
8-15	Flock 1 vs Flock 2	-0.032	0.005	*< 0.001*	**< 0.001**
	Control vs UVA	-0.035	0.009	*< 0.001*	**0.004**
	Control vs UVA +UVB	0.018	0.005	*< 0.001*	**0.017**
15-20	Control vs UVA	-0.070	0.014	*< 0.001*	**< 0.001**
15-22	Control vs UVA +UVB	0.063	0.015	*< 0.001*	**< 0.001**
20-27	Control vs UVA	-0.025	0.013	*0.051*	0.510
	Control vs UVA +UVB	0.036	0.009	*< 0.001*	**0.002**
27-34	Control vs UVA +UVB	0.091	0.012	*< 0.001*	**< 0.001**
(Age)	**FEMALES**				
8-15	Flock 1 vs Flock 2	-0.049	0.006	*< 0.001*	< 0.001
	Control vs UVA	0.013	0.007	0.065	1.555
	Control vs UVA +UVB	0.018	0.010	0.059	1.421
15-20	Control vs UVA	-0.101	0.027	*< 0.001*	**0.003**
	Control vs UVA +UVB	-0.110	0.028	*< 0.001*	**0.001**
15-22	Control vs UVA	-0.020	0.012	0.096	0.480
27-34	Control vs UVA	0.079	0.015	*< 0.001*	< 0.001
	Control vs UVA +UVB	0.089	0.033	*0.009*	0.137

End Weight (g)	Estimate	Standard error	p	Adjusted p value
MALES				
Flock 1 vs Flock 2	-0.026	0.006	*< 0.001*	**< 0.001**
Age effect	0.023	0.001	*< 0.001*	**< 0.001**
Control vs UVA +UVB	0.085	0.035	*0.015*	0.402
Age effect (UVA + UVB)	-0.002	0.001	*0.021*	0.556
FEMALES				
Age effect	0.021	0.000	*< 0.001*	**< 0.001**
Control vs UVA	-0.014	0.008	0.071	1.974

1.4) DISCUSSION

The findings presented here (summarised in Table 1.4) suggest that UV wavelengths do not negatively impact the growth, breast weight, or leg weights of male or female broiler chickens. Improvements in the average daily gains of broilers in the UVA + UVB treated group indicate UV wavelengths may have the potential to improve the growth and performance of male broiler chickens. Broiler chickens in the UVA treated group had slower growth rates though end weights were not affected. Mortality was not affected by either light treatment.

Table 1.4 – Summary of the impacts of UVA and UVB wavelengths in the current study,

Male broilers in the UVA + UVB treatment had increased growth between day 8-34. There was no significant effect of the lighting treatments on end weights of broilers. Broilers in the UVA only treatment had decreased weight gain during the earlier stages of growth. However, female broilers had increased weight gain between 27-34 days old compared to control females. There were no significant differences in the day 34 weight or end weights of UVA treated broilers compared to control broilers. There was no effect of UV wavelength supplementation on mortality or breast or leg weights.

Performance Indicator	Impact summary	
	UVA (18 hrs)	**UVA + UVB (8 hours)**
Weekly Weights	reduced in females (8- 20 days) and males (8-27 days)	increased in males (day 8-34) reduced in females (day 8-20)
Average Daily Gain	reduced in males (8-20 days) and females (day 15-22) during early growth. Increased in females during later growth (day 27-34)	increased for males (8-34 days) reduced in females (15-20 days)
End Weight	No impact	No impact
Mortality	No impact	No impact
Breast weight	No impact	No impact
Leg weight	No impact	No impact

1.4.a) Mortality

The rapid weight gain of broiler chickens is linked to increased susceptibility to cardiac arrhythmia and Sudden Death Syndrome, further highlighted by studies demonstrating early feed restriction decreases mortality in broilers (Bowes, et al., 1988; Olkowski, et al., 1997; Olkowski & Classen, 1998). Broilers in the UVA treated group had reduced growth between 8- 22 days of age, which may have contributed to the observed reduction in mortality in this group compared to the control (75% reduction in mortality). However, there was no significant effect of lighting treatment on the mortality of broiler chickens in either treatment.

Stress plays a key role in the pathogenesis of Sudden Death Syndrome (Olkowski, et al., 2008), and as UVA lighting has been found to increase exploratory behaviours and reduce baseline stress levels in young chicks (Maddocks, et al., 2001), therefore it is possible that UVA light supplementation may reduce mortality through modulating the stress response, however this is not supported by the current study and further investigation would be required to investigate the UV dose necessary to achieve this effect. Maddocks et al., provided UVA light using a halogen security light which has a different spectral composition to the lights used in the current study.

One of the limitations of the current study is the lack of males randomly allocated to the UVA only treatment from the hatchery. Male broiler chickens are generally more susceptible to Sudden Death Syndrome (Olkowski & Classen, 1998) and as the sex of all mortalities during the trial was not recorded, it is not possible to say if this effect was observed in the current study. It is possible that the differences in mortality simply reflect the differences in sex ratios of the treatments. Post-mortems were also not performed on all mortalities and would be an important consideration for any future investigation of the effects of UV wavelengths on mortality.

1.4.b) Growth, Final Weights and Breast and Leg Weights

Supplementary UVA and UVB wavelengths affected the weights and growth of broilers differently for males and females at various stages of the experiment. The provision of localised patch of UVA and UVB for 8 hours, using a fluorescent light source, increased the weight and average daily gains of male broilers throughout the 8-34 day growth period. There was no impact of either treatment on the end weights of broilers.

Based on studies investigating vitamin D metabolites on broiler chickens (Yarger, et al., 1995; Fritts & Waldroup, 2003; Fritts & Waldroup, 2005), and UVB light (Edwards, 2003), it was hypothesised that providing a combination of UVA and UVB for 8 hours of the photoperiod would improve growth, end weights and breast weight yield.

In the current study, improved growth was observed only in male broiler chickens, which is consistent with results obtained by Edwards (2003) (which only used male broiler chickens). The lack of improvement in females may reflect characteristic sex differences between circulating hormone levels (Harvey, et al., 1979; Scanes, et al., 1984), which also regulate the hydroxylation of vitamin D in birds (Tanaka, et al., 1976).

In contrast, the provision of only supplementary UVA wavelengths for the full 18-hour photoperiod using an LED light source generally decreased the weight gain of male and female broiler chickens during the early stages of growth. However, female broilers had increased weight gain between 27-34 days old compared to control females and this later improvement in weight gain appeared to compensate for the earlier slower growth, as there were no significant differences between the weights of 34-day old broilers in the UVA only treatment compared to controls and no significant difference in final end weights throughout the growth period.

The earlier slower growth could be due to increased activity, which has been found to be promoted by UVA wavelengths in previous studies in laying hens

and broilers (Jones, et al., 2001; Maddocks, et al., 2001; Kristensen, et al., 2007; Ruis, et al., 2010; Bailie, et al., 2013).

Blue and green monochromatic or mixed colour LED light treatments have been found to increase growth and breast muscle yields together with improved feed efficiency in broiler chickens (Rozenboim, et al., 2004; Cao, et al., 2008; Cao, et al., 2012; Pan, et al., 2014; Yang, et al., 2016), yet these effects were not observed in the UVA only treatment of the current study, which mixed white and UVA LED light in addition to an increase in visible blue and violet wavelengths. There were also no improvements in breast meat yield observed in either UV treated group despite other improvements in growth in the UVA + UVB treated group. There are a range of possible explanations for this effect.

Firstly, violet and ultraviolet wavelengths may have distinct impacts on young broiler chickens, and the effects of blue and green light may not be characteristic of all short wavelengths visible to broilers chickens.

Secondly, a wide range of environmental, genetic and nutritional factors influence the growth, performance and carcass composition of broiler chickens, which may have variable interactions with lighting parameters. The studies that showed improved growth and breast yield using green and blue LEDs employed different husbandry strategies and broiler strains, some kept at much lower stocking densities than those used in commercial practice. The commercially representative stocking density ($33kgm^2$) of the current study or use of a shorter photoperiod (18 hours instead of 23 hours) may have limited the potential for improved growth or breast meat yield compared to smaller scale trials (W. A. Dozier, et al., 2005; Dozier, et al., 2006; Lewis, et al., 2009; Olanrewaju, et al., 2018).

1.4.c) Conclusion

UV did not negatively impact the overall end weights of broiler chickens. Broiler chickens provided with UVA for the full 18-hour photoperiod had slower growth than control broilers. There was an increase in the growth of male broilers reared with supplementary UVA + UVB for 8 hours, indicating the potential for UV to improve the growth performance of males, potentially reaching finishing weights sooner which is beneficial for production.

The benefits associated with green and blue monochromatic light environments were not observed in the current study using mixed white and UVA LEDs. This indicates the effects of short wavelengths may not be generalised to violet and ultraviolet wavelengths, or that these results are also influenced by other husbandry and management factors that may be limiting in the current study.

Identifying husbandry strategies that lead to improvements in broiler performance is important, but it is important to establish these improvements do not exacerbate health issues associated with rapid growth. Therefore, the next chapter will examine the impacts of UVA and UVB wavelengths on key welfare indicators in broiler chickens.

CHAPTER TWO

2) THE EFFECT OF SUPPLEMENTARY ULTRAVIOLET WAVELENGTHS ON WELFARE INDICATORS

2.1) INTRODUCTION

Birds have different visual capacities and spectral sensitivities to humans and are able to perceive UVA wavelengths invisible to the human eye (Waldvogel, 1990; Goldsmith, 2006). Many birds, including domestic poultry, also possess feathers that reflect UVA wavelengths (Prescott & Wathes, 1999b; Mullen & Pohland, 2008; Bartels, et al., 2017).

The presence or absence of UV wavelengths and the UV reflective properties of bird's feathers influence foraging behaviour (Church, et al., 1998; Siitari, et al., 2002; Honkavaara, et al., 2004), mate selection (Maddocks, et al., 2002; Griggio, et al., 2010), nestling resource allocation (Jourdie, et al., 2004; Bize, et al., 2006; Tanner & Richner, 2008), and the recognition of brood-parasite eggs (Šulc, et al., 2016).

The domestic fowl is sensitive to UVA wavelengths as low as 360nm (Prescott & Wathes, 1999; Osorio, et al., 1999). Despite this, standard industry practice for broiler chickens in the UK is indoor housing with no exposure to UV or natural light throughout the whole rearing period.

While windows may be incorporated into poultry houses, glass does not typically transmit any UVB wavelengths of light and limits the transmission of UVA wavelengths depending on the type of glass used (Duarte, et al., 2009). Consequently, light from windows may not be representative of sunlight or appear "natural" to a chicken.

UVB wavelengths (290-320nm), while not visible to chickens, may offer health and welfare benefits through supporting the endogenous synthesis of vitamin

D, which plays an important and well-established role in calcium metabolism (Matos, 2008; Stanford, 2006). Non-infectious skeletal deformities represent a significant welfare problem in commercially reared broiler chickens (Knowles, et al., 2008). Lameness is predominantly associated with selection for rapid growth rates (Julian, 1998), though may be influenced by both genetic and environmental parameters including disease status, flock management and nutritional deficiencies, Including Vitamin D deficiencies (Waldenstedt, 2006; Kapell, et al., 2012;).

Both dietary vitamin D supplementation (Ledwaba & Roberson, 2003; Whitehead, et al., 2004; Gómez-Verduzco, et al., 2013), and UVB wavelength provision have been found to support the skeletal development and bone mineralisation of chicks (Tian, et al., 1994; Edwards, 2003; Fleming, 2008;). As such, UVB provision may lead to improvements in walking ability.

Improvements in mobility not only allow birds to access resources that are essential to meet their basic needs (food and water) but may also allow birds to express normal behaviours that would otherwise be prohibitively energetically expensive (Weeks, et al., 2000).

While good physical health is arguably the foundation of good welfare, animal welfare science is continuing to address the viewpoint that good health, or the absence of suffering, is not the only factor that must be considered when establishing good animal welfare. Public attitudes to farm animal welfare has driven increased interest in measures of an animals Quality of life, and the balance between positive and negative welfare states to determine if they have experienced a "life worth living" or a "good life" (FAWC, 2009; Mellor, 2016). Therefore, while there are many different definitions and interpretations of animal welfare (Fisher, 2009), there is an emerging consensus that animal welfare relates what animals feel or their emotional (affective) states (Mendl & Paul, 2017). In practice this also creates a need to investigate and promote animal husbandry practices that facilitates animals "wants" rather than simply meeting basic needs (Dawkins, 2003).

Exposure to UV in sunlight stimulates beta-endorphin production in humans creating a sense of well-being and relaxation, relieving pain and promoting wound healing (Sprouse-Blum, et al., 2010; Slominski, et al., 2012). While the subjective emotional responses of animals in response to UV is hard to determine, their preferences when offered choices between different lighting environments, or changes in behavioural expression in different light environments, can be measured and used to make judgments about how their welfare is affected.

Widowski, et al. (1992) found laying hens had a preference for fluorescent light over incandescent light; Prayitno, et al. (1997a) found broilers reared for 28 days under a single light colour showed a preference for blue wavelengths when subsequently offered a choice between blue, green, red or white light; with the exception of those raised in blue light, which preferred the green light environment. Another study by Prayitno et al. (1997b) found broiler chickens were more active and aggressive in red light and were calmer in blue light.

Evidence suggests that UVA may be an important component of avian visual feedback, which improves temporal resolution (Rubene, et al., 2010) influences activity levels and behavioural expression (Kristensen et al., 2007; Ruis et al., 2010) exploratory behaviours (Maddocks, et al., 2001) and mate choice (Jones, et al., 2001). Therefore the provision of these wavelengths could be considered a valuable form of environmental enrichment (EE) based on the definition of Shepherdson (1998) of EE as ''an animal husbandry principle that seeks to enhance the quality of captive animal care by providing the environmental stimuli necessary for optimal psychological and physiological well-being''.

Ross, et al., (2013) found a preference for light environments containing UV across a range of bird species from varying ecological habitats; though surprisingly few studies have assessed the impacts of artificial lighting regimes including UV wavelengths on the welfare of chickens.

Ruis, *et al.* (2010) conducted a series of experiments in laying hens reared under different optimised lighting conditions and found several positive outcomes with UVA: increased preening and ground pecking, reduced fearful behaviour and reduced gentle feather pecking. Similarly, Kristensen, *et al.* (2007) showed that six-week old broiler chickens performed more preening, object manipulation, foraging, and walking when reared in lighting conditions that included UVA. Maddocks, et al., (2001) found significantly lower baseline levels of corticosterone in chicks along with a trend for increased exploratory behaviours when provided with UVA.

However, not all outcomes were positive; when laying hens were reared to 50 weeks, Ruis et al (2010) found that UVA increased incidence of severe feather pecking at certain ages which was reduced (in all lighting treatments) after the introduction of substrate (Ruis et al., 2010). Therefore, Ruis, et al., (2010) proposed that UVA may have made the feathers of conspecifics look more appealing than in standard lighting, attracting more severe pecking in an environment lacking other stimuli. This idea is also supported by results of Sherwin, et al. (1999) who observed reductions in injurious pecking in turkey poults reared with supplementary UVA in conjunction with other forms of EE. Interestingly, another study found that broiler breeder hens spent longer observing cockerels illuminated with UVA, mated more frequently and had increased locomotion compared broiler breeders in standard lighting, supporting this idea of enhanced interest in feathering, and again emphasising the importance of considering the impact of age or maturity (Jones, et al., 2001).

Together, the studies discussed above suggest that whilst UVA provision alone may not be a "quick fix" for welfare problems such as feather pecking, UVA may improve the quality and potentially the reliability of visual feedback as perceived by poultry, enhancing the appearance of both conspecifics and their environment. The evidence reviewed suggests that unless animals are housed in otherwise impoverished environments, UVA wavelengths could potentially

promote the expression of normal behaviours and reduce injurious or severe feather pecking.

In the current study three welfare indicators were measured to investigate the effects of UVA and UVB wavelengths on ROSS 308 broiler chickens: feather condition, tonic immobility and gait score. Feather condition was assessed as the growth of feathers is important in commercial settings to provide birds with protection from injury and for thermoregulation (Leeson & Walsh, 2004a; Leeson & Walsh, 2004b). Feather growth and feather quality are impaired by both exogenous administration and environmental stress-induced endogenous production of corticosterone (DesRochers, et al., 2009; Lattin, et al., 2011). Plumage condition has also been associated with indicators of stress and fearfulness such as tonic immobility duration and blood leukocyte ratios (Campo, et al., 2001; Campo, et al., 2007; Campo & Prieto, 2009). This makes feather condition an interesting parameter to investigate in conjunction with other welfare measures.

Tonic Immobility (TI) duration has been proposed as a useful measure of fearfulness, an adaptive anti-predator response which is increased in more fearful birds (Gallup, 1979; Jones & Faure, 1981). Broiler chickens exhibiting shorter tonic immobility duration have been found to have improved growth performance and higher adaptability to stress (Wang, et al., 2013). TI duration is responsive to circulating stress hormones and increases following corticosterone administration (Jones, et al., 1988) or in response to stressors such as continuous lighting (Campo, et al., 2007), or noise (Campo, et al., 2005). TI duration is also shorter in birds provided with environmental enrichment (Jones & Waddington, 1992), and thus it would be predicted that UVA wavelengths may reduce tonic immobility duration.

Lastly the Bristol Gait Score developed by Kestin, et al. (1992) is a validated scoring system used to evaluate the walking ability of broiler chickens. Higher scores where mobility is compromised are indicative of poor welfare. The provision of UVB wavelengths may support skeletal development and bone mineralisation, (Tian, et al., 1994; Edwards, 2003; Fleming, 2008) potentially

leading to improvements in walking ability. Similarly, as UVA has been shown to encourage activity in broilers, the increased mechanical loading of the skeleton associated with higher activity levels may contribute to improvements in walking ability (Foutz, et al., 2007b).

The aim of the study was to investigate the impact of UVA and UVB wavelengths on feather condition, fearfulness and walking ability. It was hypothesised that UVA provision would reduce fearfulness and that both UVA and UVB provision could potentially improve walking ability (table 3.1).

Table 2.1 – summary of hypothesised impacts of UVA and UVB wavelengths, Based on the findings of previous studies UVA may reduce fearfulness and both UVA and UVB provision could potentially improve walking ability.

Welfare Indicator	Potential impact	
	UVA	UVB
Feather condition (24 days)	unknown	unknown
Tonic immobility duration	decreased (reduced fear)	unknown
gait score	decreased (improved)	decreased (improved)

2.2) METHODS

Details of the Animals used, general husbandry procedures, lighting equipment, and wavelength composition of the light treatments can be found in section VI.

2.2.a) Feather score

The feather condition of all the birds (n = 546) was assessed when they were 24 days old. Feather cover is not as commonly assessed in broiler chickens as it is in laying hens, where plumage cleanliness is more often scored for broilers at the point of slaughter (Bock & de Jong, 2010). Feather covering to assess the impacts of dietary energy level and stocking density has been performed at 21 (Škrbić, *et al.,* 2009) and 28 days of age (Moreira*, et al.,* 2006) as this falls within the period of rapid feather growth for broiler chickens (Moran, 1981). Therefore, scoring at 24 days of age was conducted for this study as it fell within this range and fit in well with other data collection points.

At this time point, 72 broilers had been culled at 9 (n =36) and 21 (n=36) days old to assess development and there had been 20 mortalities. The RSPCA feather score index (Table 2.2.a) was used as an indicator of feather cover during development. This is a four-point scale of 0, 0.5, 1, 1.5 and 2 assigning birds a score of feather coverage from a score of "full and even over body and wings" (0) to "bare on the body and patchy on the wings" (2) (RSPCA, 2013).

Table 2.2.a- RSPCA feather scoring scale for assessment of feather coverage

All broiler chickens were examined at 24 days old and assigned a score of 0, 0.5, 1, 1.5 or 2 (RSPCA, 2013).

Score	Description of Feather score
0	Feather cover is full and even over body and wings
0.5	Feather cover is slightly patchy on the sides OR back of the body OR on the wings
1	Feather cover is patchy to bare on the sides or back of the body
1.5	Feather cover is patchy to bare on the sides of the body with a light covering on the back
2	Body is bare of feathers and wings are patchy of feathers

Statistical Analysis- Feather Score

Due to lack of variation in the results of female broilers (n=9 scores of ≥1) only males' results were analysed (n = 245 treatment A : n = 21, treatment B: n = 112 treatment C: n = 112,). A generalised linear model (glm) was constructed to investigate the impacts of multiple independent variables (weight, flock and lighting treatment) on the dependent variable of Feather Score in R statistical software. "Flock" was not included in the model for this analysis due to the small sample size of males in Flock 2, Treatment A (n =2). Only 19 males obtained feather scores of 1.5, so these scores were combined with scores of 1 in to a single category, giving a binary outcome of birds scoring 0.5 (better feathered) or ≥1 (worse feathered). "Lighting treatment" and "Weight at 27 days old" were included as independent variables in the final model.

2.2.b) Tonic Immobility duration

Tonic Immobility (TI) is an adaptive anti-predator behaviour used as an indicator of fearfulness in chickens. TI duration was measured from 50-53 birds per room (n = 302, Treatment 1, n = 100, Treatment 2, n = 101, Treatment 3, n =101) at 29 days of age. An area of the pen was sectioned off with opaque boards to allow birds to be individually assessed out of sight of their flock mates. Each bird was gently restrained on their right side on a changing mat, which could be wiped down between birds if needed. The bird was gently restrained with the left wing held closed against the body for 30 seconds to induce TI. This was attempted a maximum of three times, after which a score of zero was awarded. The number of induction attempts was recorded and the duration of TI was timed using a digital stopwatch for a maximum duration of 180 seconds, after which any birds remaining in T.I were gently righted and returned to the main flock.

Statistical Analysis- Tonic Immobility

A generalised linear model (glm) with poisson family (analogous to a logistic regression) was constructed to investigate the impact of multiple independent variables (Sex, weight, flock, lighting treatment, time of test, handler inducing T.I) on the independent variable, "Tonic Immobility time". Ordered logistic regression (polr) was performed to investigate the effects of the same independent variables on an ordered categorical dependent variable "Tonic Immobility induction attempts" (recorded for n =272 tests), in R statistical software. A further glm with binomial family (analogous to a logistic regression) was performed to investigate the effects of the same independent variables on the likelihood that birds obtained the maximum time of 180 seconds (binary dependent variable). In all models, "Flock" was included to control for data being collected at different time points as Flock 1 and Flock 2 were a week apart in age (table 2.2.d).

2.2.c) Walking ability

Walking ability was assessed for n = 293 birds when they were 31 days old. Under commercial conditions birds may be "thinned" at earlier slaughter ages. Therefore in field conditions gait scoring may be conducted at a range of ages from 28-42 days (Cordeiro, et al., 2009), 28-56 days(Knowles, et al., 2008) 35 days (Kestin, et al., 1999) and 39-42 days (Dawkins, et al., 2004). 31 days of age was chosen in the current study as it is within this range and fit in well with other scheduled data collection. All birds were observed by the same two handlers, who agreed upon a score based on the Bristol Gait Score criteria (table 3.2.c) established by Kestin, et al. (1992). The Bristol Gait Score is a six - point scale from a score of zero (describing smooth fluid locomotion) to a score of five (where the bird is unable to move). A score of three or higher is considered indicative of compromised welfare and commercially birds obtaining these scores are culled (also in this study). No birds had been culled due to compromised walking ability before gait scoring was carried out. The front of each pen was sectioned off with opaque boards to create a runway of 2.5 meters. Each individual bird was placed at the end of the run-way and encouraged to walk away from the handler to the other side of the pen where a gap was left to allow it to re-join the flock.

Statistical Analysis- Walking ability

Ordinal logistic regressions analysis (polr) was performed in R statistical software to investigate the effects of multiple independent variables (Sex, weight, flock, lighting treatment and interaction effects between these variables) on an ordered categorical dependent variable; "Gait Score". A single outlier was removed from the data set (one bird in the control treatment that received a gait score of 4). After backwards elimination of non-significant terms "Treatment", "Flock", "Weight at 27 days old" and an interaction effect between "Weight" and "Treatment" were the only remaining independent variables in the model.

Table 2.2.c- Bristol Gait Score criteria (Kestin, et al., 1992) describing walking ability

Score	Description
0	The bird displays smooth, fluid locomotion. Typically, the foot is picked up and put down smoothly and each foot is brought under the bird's centre of gravity as it walks (rather than the bird swaying). Often, the toes are partially curled while the foot is in the air.
1	The bird has a slight defect in its gait that is difficult to define precisely. The bird may take unduly large strides, be unsteady or wobble when it walks, which produces an uneven gait, but the problem leg is unclear/cannot be easily identified.
2	The bird has a definite and identifiable gait abnormality, but this does not affect its ability to move. The bird may make short, quick, unsteady steps with one leg, but is not sufficiently lame to seriously compromise its ability to move, i.e. manoeuvre, accelerate and run.
3	The bird has an obvious gait defect that affects its ability to move (bird welfare is compromised) The bird may have a limp, jerky or unsteady strut, or splay one leg as it moves. The bird often prefers to squat when not coerced to move and will not run.
4	The bird has a severe gait defect. The bird is capable of walking, but only with difficulty and when driven or strongly motivated. Otherwise it squats down at the first available opportunity.
5	The bird is incapable of sustained walking on its feet. Although it may be able to stand, the bird cannot walk except with the assistance of the wings or by crawling on the shanks

Independent variables and interactions between them were included in ordered logistic regression tests (for categorical dependent variables n = 3 models) or generalised linear models (for binary dependent outcomes n = 2 models) to determine their effects on the welfare indicators of interest; feather score, tonic immobility duration and Bristol gait score.

Independent variables	Dependent variables
Sex	Feather score (binary)
Flock	Tonic immobility duration (continuous)
	Number of TI inductions required (categorical ordinal)
Lighting treatment	
Weight (27 days old)	Likelihood of 180 sec T.I duration (binary)
	Bristol gait score (categorical ordinal)

2.2.e) Corrections for multiple testing

Corrections for multiple testing were performed for all above models using a modified Bonferroni procedure (Haccou, et al., 1992). Models were ordered by p value from lowest to highest. P values were then adjusted by multiplying all p values in each model by the total number of models (n = 5) for the first model, and then subsequently multiplying by n - 1 (for the model with the second lowest p values) followed by n- 2, then n- 3, until the p values of the model with the highest p values were multiplied by n-4 and thus stayed the same. Unless otherwise stated, corrected p values are presented within text and figures.

2.3) RESULTS

2.3.a) Feather Score

Before corrections for multiple testing twenty-four-day-old male broiler chickens had better feathering in the UVA only treatment compared to the control treatment (A vs C glm: n=245, z = -2.16, p = 0.031) and there was a trend for males to have worse feather scores in the UVA + UVB treated group (B vs C glm: n= 245, z = 1.85, p = 0.06) (figure 3.3.a). However, these effects were not significant after corrections for multiple testing (A vs C glm: n=245, z = -2.16, p = 0.122) (B vs C glm: n= 245, z = 1.85, p = 0.259). Weight had a significant impact on feather score, with heavier birds having poorer feathering than lighter birds (glm: n= 245, z= 4.05, p <0.001).
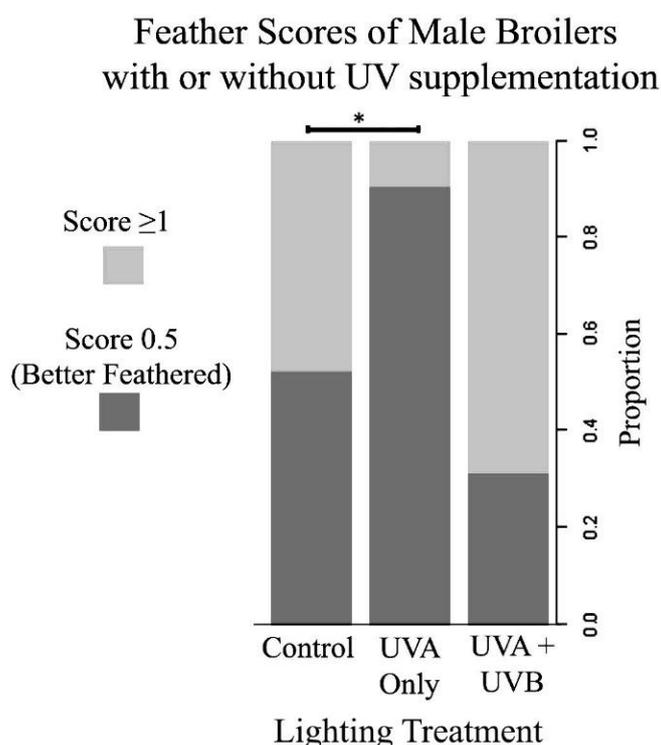
Figure 2.3.a – Feather scores of male broiler chickens
Proportion of male broiler chickens in each lighting condition obtaining scores of 0.5 (better feathering) or ≥1 (worse feathering). Before corrections for multiple testing male broiler chickens had significantly better feathering in the UVA only treated group* (p = 0.031) compared to controls and there was a trend for males in the UVA + UVB treated group to have worse feather condition than control males (0.065). However, these results were non- significant after corrections for multiple testing.

2.3.b) Tonic Immobility duration

Broiler chickens in the UVA only treatment had shorter T.I durations than the control group (C vs A glm: n=302, z = -3.14 p = 0.003, figure 2.3.b). and were less likely to obtain the maximum time of 180 secs (C vs A glm: n= 302, z= -3.14, p = 0.005), though more likely to require multiple T.I induction attempts (C vs A: polr: n= 272, z = 2.31, p = 0.021) than control broilers. Tonic immobility time was not significantly different to the control treatment in the UVA+ UVB treated group. There was no significant effect of different handlers, the time of day the test was performed, sex, weight or flock on T.I induction or duration before or after corrections for multiple testing.
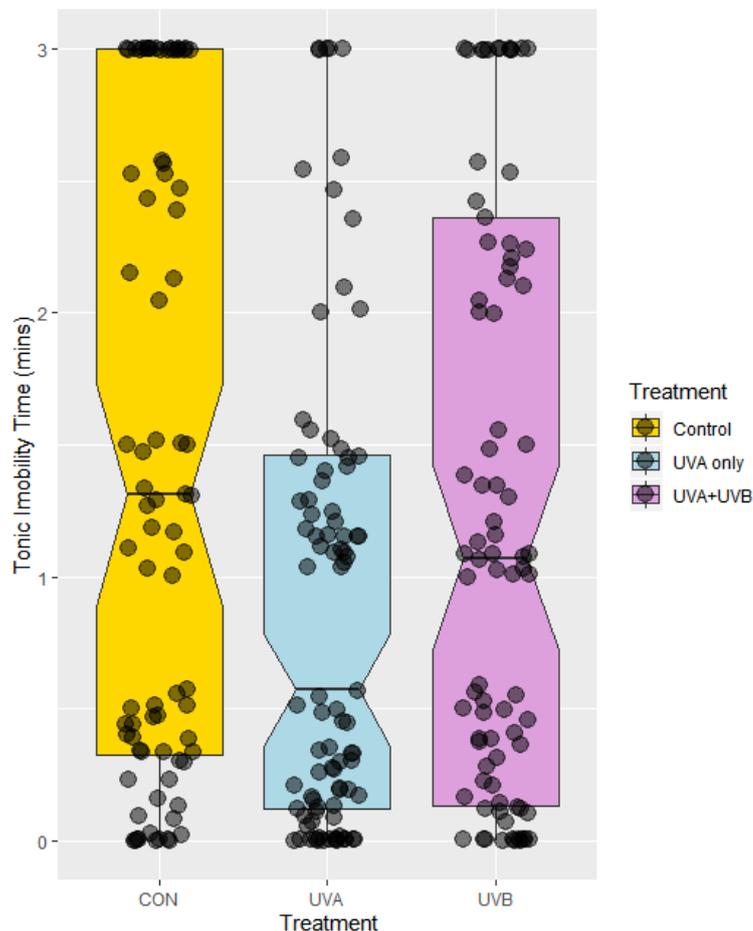
Figure 2.3.b) Broiler chicken's tonic immobility (TI) duration (mins) with and without UV supplementation.
Broilers in the UVA only condition had significantly shorter TI durations compared to control birds. They were also significantly less likely to obtain the maximum TI duration of 180 seconds.

2.3.c) Walking ability

Broiler chickens with UV wavelength supplementation had improved walking ability compared to control broiler chickens (Figure 3.3.c and table 3.3.d). Gait Scores were significantly lower (better) in the UVA + UVB condition (polr: n=293, z = -229.32, p <0.001) and the UVA only treated group (polr: n = 293, z = -1158.18, p < 0.001).   Heavier birds had higher (worse) gait scores (polr: n=293, z= 24.21, p= <0.001), and there was a significant interaction between weight and treatment. Heavier birds in the UVA only (A vs. C: polr: n =293, z= 20.86, p= <0.001) and the UVA+ UVB treatment (B vs. C polr: n= 293, z= 7.13, p= <0.001) had lower gait scores than control birds of similar weights.
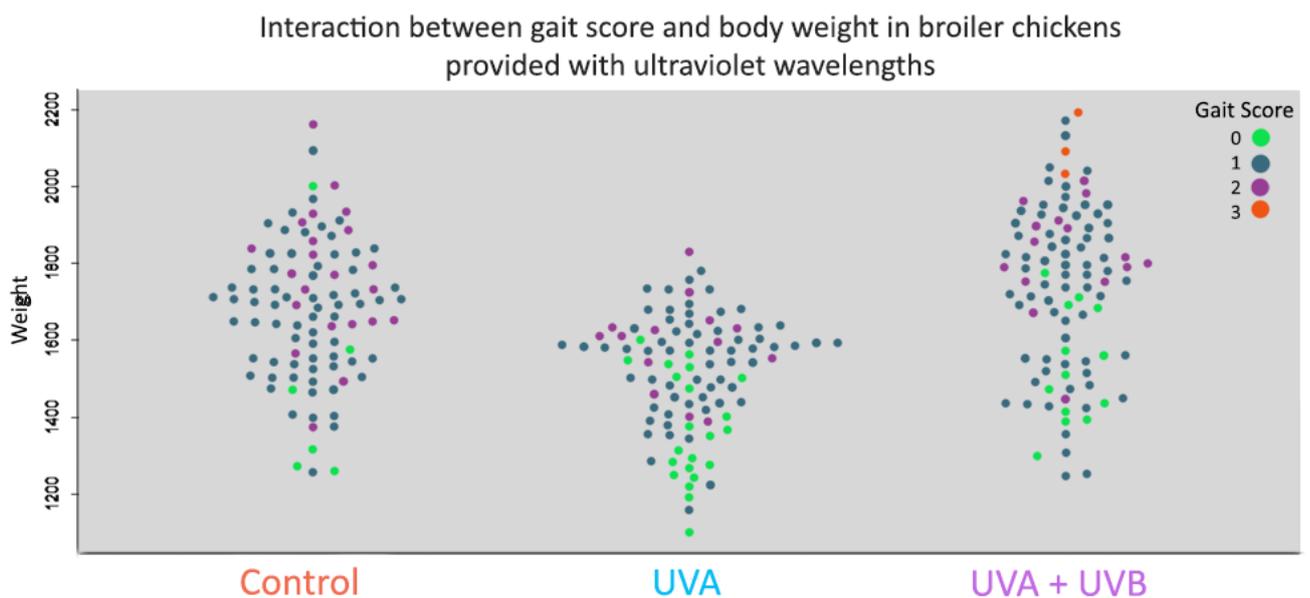
Figure 3.3.c) gait scores and weights of broiler chickens with and without UV supplementation.
Gait score was lower in the UVA only (p < 0.001) and UVA+UVB (p < 0.001) treated group. Additionally, there was a significant effect of weight, with heavier birds more likely to obtain higher gait scores (p< 0.001), and an interaction effect between weight and treatment, with birds in the control group generally having higher gait scores compared to birds of similar weights in the UVA (p < 0.001) and UVA + UVB treated group (p < 0.001).

Table 2.3.d) – Odds ratios and 95% confidence intervals or model estimate and standard error for broiler chicken welfare indicators with and without supplementary UV wavelengths.

Outcomes of ordered logistic regression[1] or generalised linear models[2]. Significant results (p < 0.05) before corrections for multiple testing are italicised. Significant outcomes after adjustment of p values using the modified Bonferroni procedure (section 2.2.e) are emboldened. Exposure to supplementary UVA only (Treatment A), reduced tonic immobility (TI) duration and reduced the likelihood of broilers obtaining maximum TI times. Broilers in the UVA only condition were more likely to require multiple TI induction attempts and had better gait scores compared to control broilers. Broilers exposed to UVA + UVB also had improved gait scores. Increases in weight significantly worsened feather score and gait score, though there was also an interaction between weight and treatment on walking ability, with heavier birds in both UV treated groups having improved gait scores compared to control broilers of similar weights.

	Odds ratio	95% confidence intervals		p	Adjusted p value
Male Feather Scores[2]		-	+		
Control vs UVA	0.180	0.038	0.851	*0.031*	0.122
Control vs UVA +UVB	1.731	0.967	3.101	0.065	0.259
Weight (g)	1.004	1.002	1.006	*< 0.001*	**< 0.001**
Tonic Imobility Duration		-	+		
T.I time (mins)[2]	**Estimate**	**Standard error**			
Control vs UVA	-0.401	0.128		*0.002*	**0.003**
Control vs UVB	-0.173	0.120		0.148	0.296
Flock 1 vs Flock 2	0.106	0.102		0.300	0.600
likelihood of obtaining max time (180 sec)[2]	**Odds ratio**	**95% confidence**			
		-	+		
Control vs UVA	0.324	0.160	0.654	*0.002*	**0.005**
Control vs UVB	0.561	0.298	1.056	0.073	0.220
Flock 1 vs Flock 2	1.364	0.786	2.367	0.269	0.808
likelihood of requiring multiple T.I inductions[1]					
Control vs UVA	1.983	1.109	3.545	*0.021*	**0.021**
Control vs UVB	1.457	0.854	2.486	0.168	0.168
Flock 1 vs Flock 2	0.944	0.594	1.502	0.809	0.809
Gait Score[1]		-	+		
Control vs UVA	0.001	0.001	0.001	*< 0.001*	**< 0.001**
Control vs UVA +UVB	0.053	0.051	0.054	*< 0.001*	**< 0.001**
Weight (g)	1.005	1.004	1.005	*< 0.001*	**< 0.001**
Weight effect (UVA only)	1.004	1.003	1.004	*< 0.001*	**< 0.001**
Weight effect (UVA + UVB)	1.001	1.001	1.002	*< 0.001*	**< 0.001**
Flock 1 vs Flock 2	2.083	1.293	3.355	*0.002*	**0.013**

2.4) DISCUSSION

Findings presented here (summarised in table 2.4) suggest UVA and UVB may offer potential welfare benefits to indoor reared broilers. UVA led to reduced fearfulness and improved walking ability. In the UVA + UVB treatment walking ability was also improved.

Table 2.4) summary of the impacts of UVA and UVB wavelengths
UVA led to reduced fearfulness and improved walking ability. Walking ability was also improved in the UVA + UVB treatment. There was no effect of lighting on feather condition.

Welfare Indicator	Impact summary	
	UVA (18 hrs)	UVA + UVB (8 hours)
Feather condition (24 days)	No impact	No impact
Tonic immobility duration	decreased (reduced fear)	No impact
Gait Score	decreased (improved)	decreased (improved)

2.4.a) Feather Cover

Feathering analyses were limited to males, due to lack of variation in female feather scores. The feather development of female broiler chickens is faster than males, which may explain the sex differences observed in the current study (Moran, 1981; Deschutter & Leeson, 1986; Siegel, 1963; Hancock, et al., 1995; Wecke, et al., 2017). Additionally, the RSPCA feather score, as a general assessment of whole-body feather cover, may not be sensitive enough to detect the smaller variations in feather cover that may have been present in females.

Only 4% of broilers obtained a score of 1.5 and none were given a score of 2 at 24 days old. While this could indicate feather condition in the current study was generally good, It is hard to confirm this as few studies that have assessed feathering in broilers in a standardised way. Škrbić, *et al.,* (2009) only assessed feather cover on the breast areas and Moreira*, et al.* (2006) used a different scoring system for the thigh and back region seperately. At 24 days old feather cover is expected to be within 5.0-5.2% of total body weight for males and 5.4-6.4% for females (Wecke*, et al.,* 2017). In order to draw more robust conclusions on the impacts of lighting and other management factors on feathering rate a more consistent scoring approach should be adopted for young broiler chickens.

While no significant differences in feathering were observed in the current study after corrections for multiple testing, feathering rate may still be a worthwhile area of future investigation for the following reasons. First, feather growth is energetically expensive. The rate feather growth is highest during the first 6 weeks of age (Moran, 1981; Stilborn, et al., 1994), with feathers maturing earlier than other body components (Gous, et al., 1999; Bonato, et al., 2016;). At low ambient temperatures, feather growth has been shown to be maintained in preference to, or even at the expense of, muscle development in turkeys when feed availability was restricted, suggesting feather growth takes precedence to muscle growth in terms of nutrient ultilisation (Wylie, et al.,

2001). However, this may not apply to broilers kept in much warmer temperature-controlled environments where insulation may not be as important.

As such, under natural conditions feather growth and the maintenance of feather quality is energetically costly and thought to be an indicator of an individual's condition (Hill & Montgomerie, 1994; Bortolotti, et al., 2002; Bulluck, et al., 2017; Jovani & Rohwer, 2017), and the quality of their environment (Swaddle & Witter, 1994; DesRochers, et al., 2009; Lattin, et al., 2011; Will, et al., 2014; Patterson, et al., 2015). Aspects of plumage condition are thought to act as honest signals in a variety of social contexts including mate selection (Zahavi, 1975; Hill, 1990; Hill, 1991; Siefferman, et al., 2005), signals of social status (Nakagawa, et al., 2007) and in parent-offspring communications (Tanner & Richner, 2008; Griggo, et al., 2009).

However, in captive settings, domestic fowl have been selectively bred for production characteristics and are kept in environments and social groupings that are not representative of their progenitor species (Wood-Gush, 1973). In mature laying hens, feather condition is often considered a key welfare indictor related to levels of feather pecking (Huber-Eicher & Sebö, 2001; Bestman, *et al.*, 2009; Blokhuis, *et al.*, 2007) with both perpatrators and victims potentially experiencing reduced welfare, as indicated by higher levels of fluctuating asymmetry (Tahamtani, *et al.,* 2017), and measures of fear and stress (El-Lethey, *et al.,* 2000).

However, the causes of plumage damage and feather pecking are multifactorial (Rodenburg, *et al.,* 2013; Nicol*, et al.,* 2013) which can lead to variable results depending on environmental conditions. Furthermore, plumage damage was not related to environmental choice in laying hens exposed to different environments (Nicol, *et al.,* 2009), suggesting hens themselves did not prioritise environments where their plumage condition was improved. However, levels of pulmage damage and pecking observed by Nicol *et al.* (2009) were low, suggesting that feather condition in isolation may not reflect affective states

and that the causes and severity of feather damage or loss needs to be considered when interpreting these measures.

Another consideration for future investigation is the UV reflective properties of the skin and feathers of birds; which play a role in social signalling (Doucet & Montgomerie, 2003; Keyser & Hill, 2003; Bize, et al., 2006; Sirkiä & Laaksonen, 2009; Griggio, et al., 2010; Henderson, et al., 2013) and have been found to correlate with reproductive success and corticosterone levels in other bird species (Henderson, et al., 2013).

Additionally, feather condition is also maintained through preening, and studies have demonstrated an increase in preening behaviours in chickens provided with UVA wavelengths (Kristensen, et al., 2007; Ruis, et al., 2010). The appearance of birds' feathers in the presence of UVA wavelengths may provide more accurate cues of plumage condition than standard lighting, stimulating more preening behaviours. In budgerigars UV reflectance was lower in birds prevented from preening, and females spent more time with males with a higher UV reflectance in preference tests (Zampiga, et al., 2004; Griggio, et al., 2010).

Prescott & Wathes (1999b) found no evidence of feathering patterns in domestic chickens that would be visible only under UVA. However, there was variation in the UVA reflectance of feathers on different areas of the body and variation between individuals, which may provide visual cues for use during sexual selection or other during other social interactions (Prescott & Wathes, 1999b). These cues may have influenced mate choice in the study by Jones et al. (2001). Therefore, UV reflectance cues deserve further investigation in poultry to determine their roles in social communication and if they have potential applications as an indicator of health or welfare.

2.4.b) Fearfulness

Broiler chickens provided with UVA exposure for the full 18-hour photoperiod were less fearful than control broilers, as indicated by shorter tonic immobility durations (Gallup, 1979). A similar effect was not observed in broilers provided with UVA + UVB for only 8 hours a day. This may reflect a dose-dependent effect of UVA, resulting from an experimental limitation of UVB (hence also UVA) to 8 hours a day, in treatment B. There was also a reduced spread of UVA wavelengths from the fluorescent lamp in treatment B compared to treatment A, where UVA was provided by a LED light (as shown in Figure VI.II.b). Contradictory impacts of UVB on T.I duration cannot be ruled out, though no studies currently support or refute this possibility.

The impacts of UVA observed here are in agreement with findings by Ruis, et al., (2010) and Maddocks, et al., (2001) which support the idea that UVA reduces fearfulness. More recent studies have also found the supplementation of UVA wavelengths reduces T.I duration, decreases fluctuating asymmetry and reduces heterophil lymphocyte ratios in broiler chickens (House, *et al.,* 2020) and laying hens (Sobotik, et al., 2020).

A potential explanation for this is the ability of UVA to alter neurological pathways. UVA dramatically suppress melatonin secretion via retinal perception and direct photo-stimulation of the pineal gland and is capable of phase-shifting circadian oscillators (Zawilska, *et al.,* 2007). UVA wavelegnths decrease the action of AANAT (a key enzyme involved in melatonin systhesis), in the pineal gland and the retina. This occurs via the stimulation of retinal N-methyl-D-aspartate (NMDA) glutamate receptors rather than the Dopaminergic neurotransmission associated with the retinal reception of white light (Rosiak & Zawilska, 2005). Pharmacological studies indicate stimulation with white light and UVA light may involve different classes of receptors and distinct neurotransmitter systems (Rosiak & Zawilska, 2005; Zawilska, *et al.,* 2007), which may have impacted the amplitude of circadian rhythms in the

current study, shortenting T.I duration in the UVA treated group by increasing melatonin suppression compared to the control group.

T.I durations increase in chickens following the administration of serotonin and melotonin in a dose dependent response (Hennig, et al., 1980), which may further explain why reductions in T.I times were not observed in the broilers in treatment B, which received 10 hours less exposure to UVA than those in treatment A.

However, the UVA LED used in the curent study also increased visible blue wavelengths, which have similarly been found to reduce fear levels in broiler chickens (Mohamed, *et al.,* 2017; Mohamed, *et al.,* 2020) and modulate the stress responses through a reduction of circulating interleukin-1β (Xie et al., 2008). Broiler chickens reared in monochromatic blue light were found to have lower amplitude in circadian melotonin production compared to other light conditions and increased serotonin levels (Jin, et al., 2011). Therefore the current study design does not truly allow for the distinction bettween wether true UVA wavelengths, blue wavelengths or a combination of both, may be associated with the observed effects.

UVA may also have the potential to reduce stress or fearfulness indirectly. The performance of highly motivated behaviours such foraging and dustbathing are thought to be inherently rewarding, with frustration and abnormal behaviours occurring where these behavioural needs are not met (Duncan, 1998; Weeks & Nicol, 2006). Increasing environmental complexity, through the introduction of appropriate litter, elevated platforms or the provision of straw bales, provides opportunities to meet behavioural needs can result in improvements in activity levels, reduced fear levels and improvements in learning ability in broiler chickens (Kells, et al., 2001; Brantsæter, et al., 2017; Tahamtani, et al., 2018) . However the floor space allowance, labour, time and costs associated with these forms of enrichment may make them less appealing for intensive commercial farms, who may be more likely to make one-off investment into UVA bulbs than into other forms of EE. However, UVA wavelengths may potentially enhance the appearance of, or increase engagement with, other

forms of environmental enrichment (EE) leading to additive effects of fear or stress reduction.

Further research could also determine if the provision of UVA may potentially eradicate fear and stress associated specifically with the ambiguity of visual feedback in environments lacking UVA.

2.4.c) Walking Ability

Walking ability, assessed using the Bristol gait score criteria, was improved in both UV treatments. Heavier birds were more likely to obtain worse gait scores, which is consistent with the expectation that carrying more weight should impact on mobility, and results of previous studies (Sørensen, et al., 1999; Su, et al., 1999; Kestin, et al., 2001; Kristensen, et al., 2006). However, there was also an interaction between weight and treatment, with heavier birds in the UV treated groups having better gait scores than control broilers of similar weights. These imporovements may represent a potential benefit for broiler chicken performance and welfare.

Bailie, et al., (2013) found the provision of natural light including UVA wavelengths, improved gait scores and increased latency to lie times in broiler chickens. However, their study design did not allow for distinction between which elements of natural light (wavelength composition or light intensity) were responsible for the results obtained. A study by Kristensen (2006) found no improvements in gait score where UVA was provided, though the main light sources used in the study were fluorescent lights with spectral compositions distinct from the LEDs that were the main light source in the current study.

There is evidence to suggest UVA may increase activity and exploratory behaviours in chickens (Maddocks, et al., 2001; Kristensen, et al., 2007; Ruis, et al., 2010; Bailie, et al., 2013). Mechanical loading is essential for the normal development of bones and tendons, and decreased activity can negatively

impact these tissues and consequently the walking ability of broiler chickens (Foutz, et al., 2007a; Foutz, et al., 2007b; Moussa, et al., 2007).

UVB light allows for the endogenous production of vitamin D and has been found to improve bone mineral density and reduce the incidence of tibial dyschondroplasia and rickets (Edwards, 2003; Fleming, 2008).

The improvements in gait score of UV treated broilers observed in the current study could potentially result from increased activity levels as a result of UVA wavelengths, or the provision of a localised area of 30 mw/cm² UVB may have been sufficient to support endogenous vitamin D production and skeletal growth.

Another possible explanation is the lower gait scores observed in the UV treated group could represent a reduction in pain. Lame broiler chickens show decreased activity (Weeks, et al., 2000), and their behaviour and walking ability is modified by the administration of analgesics (McGeown, et al., 1999; Danbury, et al., 2000). These studies indicate that, in addition to compromised mobility, lameness can also compromise welfare through chronic pain.

UV wavelengths have been found to directly reduce pain and promote wound healing (Sprouse-Blum, et al., 2010; Slominski, et al., 2012) which may contribute to improvements in walking ability. Additionally, UV wavelengths are thought to reduce stress and fearfulness in birds (Maddocks, et al., 2001; Ruis, et al., 2010), which is supported by the results of the current study, as Tonic Immobility (TI) duration was reduced in broilers exposed to UV wavelengths. Stress has been found to cause hyperalgesia in animal models of chronic pain (Blackburn-Munro & Blackburn-Munro, 2001). So it is possible UV may indirectly improve walking ability through a stress-mediated reduction in pain sensitivity.

However, this explanation is based on the assumption that the Bristol gait score also reflects a lameness "pain scale" which may not be an accurate assumption. A study by Siegel, et al. (2011) found that broilers obtaining higher gait scores did not self-select higher intakes of analgesic than sound birds. Additionally,

Skinner-Noble & Teeter (2009) found broiler chickens assigned gait scores of two or three in field tests had similar levels of well-being; as determined by energy metabolism, feed conversion, hetrophil lympocyte ratios, skeletal pathology and fear responses. Body conformation (particularly breast conformation) was the main difference associated between broilers with gait scores of two and three and not changes in basal metabolism, pathology of the sciatic nerve and surrounding muscle tissue, stress levels or fear (Skinner-Noble & Teeter 2009).

The evidence highlighted above, in addition to quantitative studies of broiler chicken locomotion and energy expenditure (Paxton, et al., 2013; Tickle, et al., 2018), indicates that (for lower scores) gait modification may be an adaptive mechanism that does not necessarily reflect increased pain or stress (Skinner-Noble & Teeter 2009; Siegel, et al., 2011).

However, this does not mean that husbandry interventions and efforts to improve the mobility of broiler chickens are unimportant to their welfare. Despite no adverse welfare affects, Skinner-Noble & Teeter (2009) observed broiler chickens with worse gait scores spent more time sitting. So worse gait scores may still reflect a greater energetic cost of locomotion (Tickle, et al., 2018), which limits their ability to express other natural behaviours (Weeks, et al., 2000) and potentially reduces their capacity to experience positive welfare states.

The Bristol gait score is a subjective method of gait assessment where, based on a "snap-shot" of observed walking behaviour, a score is assigned to reflect good or impaired walking ability. The resulting output is influenced by many separate components of the broiler chickens integrated locomotor system; including the conformation and integrity of the skeleton (Rath, et al., 1999; Skinner-Noble & Teeter, 2009; Paxton, et al., 2013), muscles and connective tissues essential for movement (Foutz, et al., 2007a; Foutz, et al., 2007b) together with the central and peripheral nervous system which controls locomotion (motor neurons) and responds and adapts to mechanical and sensory feedback (sensory neurons).

Therefore, while improvements in Bristol gait score may reflect improved walking ability, it is still difficult to separate these integrated components of the locomotory system and determine precisely how husbandry manipulations such as wavelength composition affect walking ability.

2.4.d) Conclusion

In conclusion, the provision of UV wavelengths may improve the welfare of indoor reared broiler chickens. Both the UVA only and the UVA + UVB treatments improved walking ability and UVA provided for the full 18 hours of the photoperiod reduced fearfulness. Further investigation is required to determine the biological mechanisms of fear reduction, though a potential role of UVA induced melotonin suppression via retinal or pineal stimulation may be a promising direction for further study. One of the limitations of this study is the current experimental design does not preclude the impacts resulting from additional visible blue wavelengths or a combination of UVA and blue wavelengths, so further studies investigating the capacity of both UVA and blue wavelengths to alter fear and stress responses would be valuable.

No treatment effects were observed on feather scores at 24 days old, and a lack of standardised broiler specific studies makes it hard to draw conclusions on whether the observations in this study are representative. Examining associations between the feather scores and other measures in this study may help determine if feather scoring is a useful welfare indicator in broiler chickens (explored in chapter 4). Other feather-based measures, such as UV reflectance or other indicators of feather quality may be promising areas of future research. Further investigation should also examine if lighting environments including UV wavelengths improve the quality of visual feedback as perceived by the birds as an alternative means of reducing fearfulness. This could be investigated by examining behaviours such as preening and comfort behaviours, monitoring activity levels, or by investigating responses to novel objects with and without the provision of UV.

CHAPTER THREE

3. THE EFFECT OF SUPPLEMENTARY ULTRAVIOLET WAVELENGTHS ON HEALTH INDICATORS

3.1) INTRODUCTION

The supplementation of UVB (290-320nm) lighting may offer important health benefits to broiler chickens predominantly through supporting the production of endogenous vitamin D, which plays an important and well-established role in calcium metabolism in birds (Stanford, 2006 ; De Matos, 2008). The role of UVB wavelengths in the synthesis of vitamin D in birds and the subsequent physiological effects of calcium and vitamin D are summarised in figure 3.1.

Despite its name vitamin D is a steroid hormone found in different forms throughout the body, the most metabolically active form of which is 1,25-dihydroxyvitamin D [1,25-(OH)2-D3] or calcitriol (Norman, 1987). The main target organs of calcitriol are the bones, intestine, and kidney where it acts to regulate the uptake of calcium from the duodenum and jejunum and release or deposit calcium within bone depending on the organism's levels of circulating ionized calcium. The effect of this is predominantly the regulation of calcium homeostasis and skeletal health and development (Norman & Hurwitz., 1993).

However, more recently non-skeletal functions of vitamin D relating to skin health, immune regulation, reproduction, cardiovascular health, glucose homeostasis and cell transcription and differentiation are being recognised (Rosen, et al., 2012; DeLuca, 2014 ; Rejnmark, et al., 2017). Both the vitamin D receptor and the 1α-hydroxylase enzyme which activates 25-hydroxyvitamin D [25-OH-D$_3$] or calcidiol in to calcitriol have been identified within the cells of the majority of body tissues, where the local production and action of vitamin D is involved in gene expression and transcription (Adams & Hewison, 2012; Pike & Meyer, 2014).
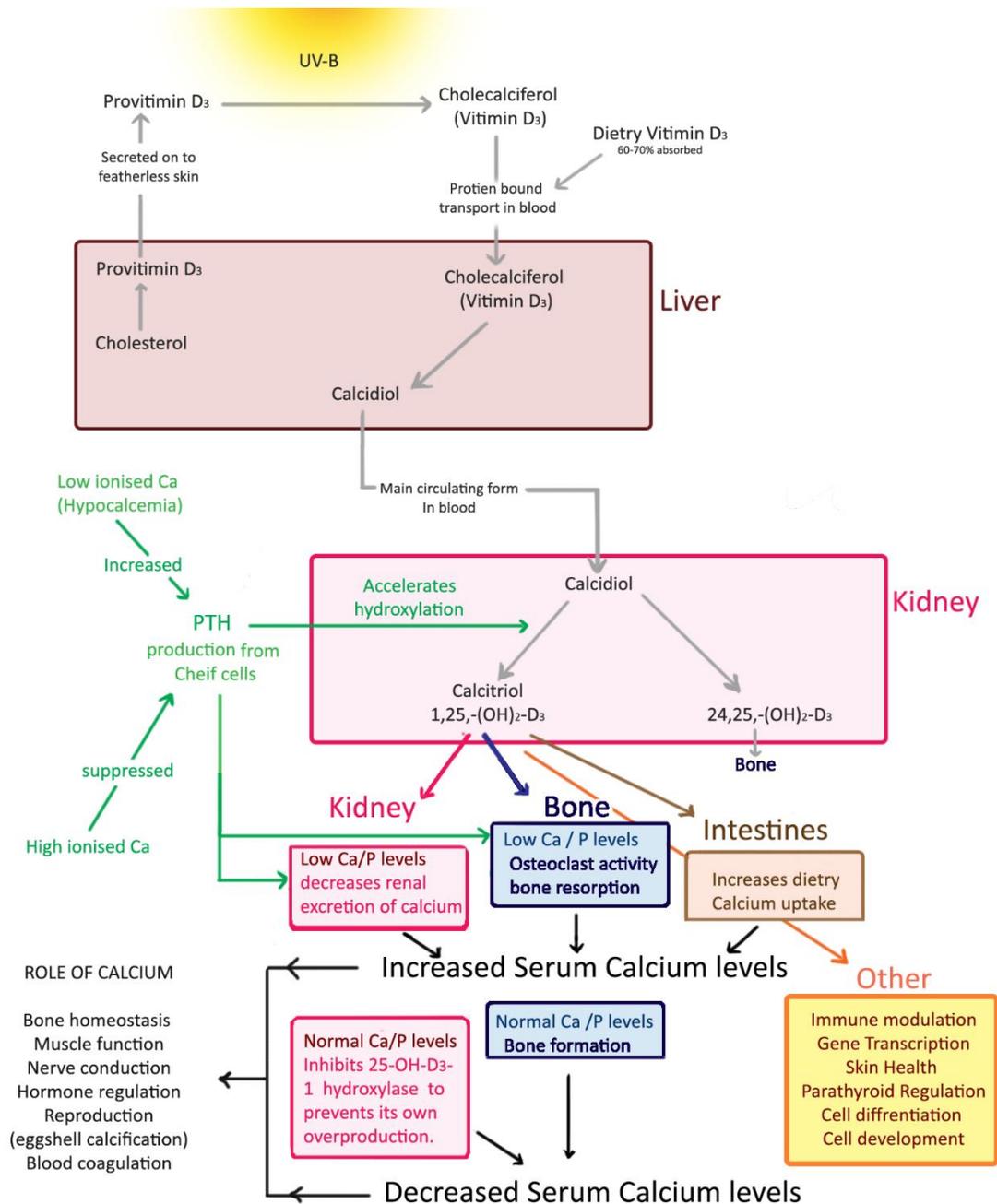
Figure 3.1- The synthesis and effects of Vitamin D in birds (adapted from De Matos., 2008)

7-dehydrocholesterol or pro-vitamin D is synthesised from cholesterol in the liver and secreted on to the skin (Tian, et al., 1994). During exposure to UVB radiation (290-315nm) provitamin D is converted to cholecalciferol. Both endogenous and dietary cholecalciferol are transported to the liver protein bound in circulation where they are converted by vitamin D-25-hydroxylase in to 25-hydroxyvitamin D [25(OH)D] or calcidiol. Calcidiol is the major circulating form of vitamin D often measured to determine vitamin D status although it is biologically inactive. Calcidiol is converted in the kidneys by the enzyme 25-hydroxyvitamin D-1α- hydroxylase (1-OHase) in a tightly regulated process to the metabolically active form 1,25-dihydroxyvitamin D [1,25(OH)2D] or Calcitriol (Norman, 1987). In hypocalcaemic conditions calcitriol acts to increases serum calcium levels through limiting the renal excretion of calcium, increasing calcium absorption in the small intestine and stimulating osteoclast activity, releasing calcium and phosphorus from the bone. Under normal levels of serum calcium Calcitriol inhibits (1-OHase) and PTH production, limiting its own production, and in these circumstances 24,25-(OH)2-D3 is the major hydroxylation product of calcidiol which is essential for chondrocyte development (Norman & Hurwitz., 1993). Bone formation is also upregulated by inducing synthesis osteoblast proteins. Together this maintains calcium homeostasis and skeletal development along with the other non-skeletal functions of vitamin D shown .

Infectious and non-infectious musculoskeletal disorders and leg weakness have significant impacts on broiler chicken health and welfare, in addition to economic consequences within the poultry industry, and have been extensively reviewed (European Commission, 2000; Bradshaw, et al., 2002; Julian, 2004; Mench, 2004; Knowles, et al., 2008).

Notable examples of disorders affecting broiler chickens include long bone deformities, which can be accompanied by slippage of the gastrocnemius tendon, bacterial chondronecrosis with osteomyelitis (BCO) and tibial dyschondroplasia (TD). TD is caused by poor vascularisation and ossification of the growth plate resulting in a cartilage mass below the epiphyseal plate. This can lead to angular and rotational deformities of the tibia, unnatural biomechanical forces and altered gait (Julian, 1998; Farquharson & Jefferies, 2000).

Non-infectious skeletal deformities, though largely associated with the rapid growth rate of broiler chickens (Julian, 1998), are multi-factorial and influenced by both genetic and environmental parameters including disease status, flock management and nutritional deficiencies, including Vitamin D deficiencies (Waldenstedt, 2006; Kapell, et al., 2012).

In the absence of UVB, indoor reared poultry are supplemented with dietary vitamin D in the form of cholecalciferol or calcidiol which have been found to reduce the incidence and severity of TD and rickets in addition to improving bone mineral density and innate immune parameters (Ledwaba & Roberson, 2003; Whitehead, et al., 2004; Gómez-Verduzco, et al., 2013; Vazquez, et al., 2017).

The absorption of dietary cholecalciferol from the gut is not as effective as the absorption of dietary calcidiol (Bar, et al., 1980). Calcidiol supplementation was found to be more effective than cholecalciferol for increasing bone ash values and body weight in broiler chickens while reducing the incidence of TD (Fritts & Waldroup, 2003). Similarly providing calcidiol in conjunction with cholecalciferol improved the tibia bone ash values and cellular immune

responses of broilers when added to the diet (Vazquez, et al., 2017), or through in ovo injection (Abbasi, et al., 2017). Broiler breeders supplemented with calcidiol have reduced embryo mortality (Saunders-Blades & Korver, 2014), and improved progeny cellular immune responses (Saunders-Blades & Korver, 2015) compared to those supplemented with only cholecalciferol. However, quantities of cholecalciferol and calcidiol used in experimental diets tend to be less than the quantities typically used in industry diets, where less differences were observed between the two precursor metabolites (Fritts & Waldroup, 2005).

The effectiveness of UVB irradiation in comparison to dietary cholecalciferol supplementation for protection against TD and rickets has also been investigated. UVB provision was more effective at improving skeletal health than dietary vitamin supplementation (Edwards, 2003; Tian, et al., 1994). Excessive levels of dietary vitamin D can also lead to Hypervitaminosis D, associated with organ and arterial mineralisation (Scott, et al., 1978), clinical signs such as anorexia, diarrhoea, dehydration, emaciation, weakness and difficulty in moving (Kumar, *et al*., 2017) and higher susceptibility to stress induced arrhythmia and sudden death syndrome (Nain, et al., 2007). Due to the self-limiting nature of endogenous Vitamin D production, this means UVB provision would eliminate these risks; though maximum safe doses for dietary Vitamin D have not been established in broilers it is important to note toxicity effects occurred when broilers were given at 16-20 times the commercially representative level of 5000 IU vitamin D3/kg (Kumar, *et al*., 2017; Nain, et al., 2007).

Continuous exposure of broiler chickens to radiation from fluorescent lights providing 9.99 mJ/s per m^2 across a spectrum of 285–365 nm at 0.15 m was found to be equivalent to 10.0–20.0 mg of dietary D3 per kg of feed (Edwards et al. ,1994). In a study by Edwards et al (2003) day old broiler chickens were exposed to mercury vapour lamps providing 856 mJ/s per m^2 across a spectrum of 285–365 nm at 0.26 m for 30-60 minutes from above or below. Illuminating broiler chickens with UVB from below was found to have a long-lasting impact

on chick development, with a greater capacity than dietary cholecalciferol to reduce the incidence of TD and rickets (Edwards, 2003).

Similarly, Tian, et al., (1994) found chickens produced up to 30 times more provitamin D on the featherless skin of their legs than on their backs when their whole bodies were exposed to UVB, indicating the importance of this area for Vitamin D metabolism.

A study by Fleming (2008) used 138 mJ/s per m^2 UVB as a treatment for chicks fed on an imbalanced Calcium, Phosphorus, and low vitamin D content diet. Bone strength was found to be improved by over 30% in the UVB treated group compared to the control group. Significant improvements were also found in tibia radiographic density and tibia ash values, suggesting that UV-treated birds had better mineralised bones and that UVB was able to compensate for imbalances in diet.

The evidence reviewed indicates UVB can have a significant impact on skeletal health. However, these studies use small pens with even UVB distribution or otherwise expose chickens to situations where they cannot move away from the UVB. Achieving even illumination of UVA in commercial settings may be possible using currently available technology, but creating even illumination of UVB across commercial poultry sheds is likely to be impractical and economically unfeasible due to the short distance artificial light sources typically transmit UVB.

However, providing smaller areas of UVB illumination could be accomplished. For example, UVB illumination could be provided by adding light fixtures to platforms provided for enrichment purposes, or to existing feeder and drinker lines across the shed. This raises further questions about the levels of UVB and distribution of illumination that might be required across an indoor poultry house to benefit the health of broiler chickens kept at commercial stocking densities.

In the current study the effects of a small area of combined UVA and UVB ($30\mu W/cm^2$ or 300 mJ/s per m^2 UVB) was investigated. Health indicators

measured were: Bone mineral density, assessed using Dual Energy X-ray Absorptiometry (DEXA), leg bone measurements, tibia breaking strength, severity of Tibial Dyschondroplasia, eye weight and cornea histology. While ocular abnormalities were not expected to result from the current study design, eye weight and cornea histology were examined to confirm this was the case for the novel lighting treatments used. Eye enlargement or Buphthalmia have been observed in response to continuous fluorescent lighting (Whitley, *et al.,* 1984), but not in exposure to continuous levels of UV from a mercury vapor lamp (Barnett & Laursen-Jones, 1976), where a roughening of the cornea was observed. However, Barnett and Laursen-Jones (1976) observed no abnormalities in the globe, periorbital region, conjunctiva, anterior segment, lens or fundus of the eye, so only the cornea was examined in the current study.

It would be expected that skeletal health measures would be improved in the UVB treated broilers if a small, localised patch of UVB was sufficient to increase vitamin D synthesis (table 2.1).

Table 3.1– summary of hypothesised impacts of UVA and UVB wavelengths,
*Based on the findings previous studies UVA and UVB may improve skeletal health indicators. * Damage to eyes is not expected to occur at the levels of UVA and UVB used in the current study.*

	Potential impact	
health Indicator	UVA	UVB
Bone mineral density	unknown	increased
Bone measurements	unknown	increased
Tibia breaking strength	unknown	increased
Tibial dyschondroplasia	unknown	reduced
eye weight	no impact *	no impact *
cornea histology	no impact *	no impact *

3.2) METHODS

Details of the Animals used, general husbandry procedures, lighting equipment, and wavelength composition of the light treatments can be found in section VI. Data were collected post-mortem to assess the following health indicators: Bone mineral density, bone measurements, tibia breaking strength, incidence of Tibial dyschondroplasia, eye weight and cornea histology.

3.2.a) Post-mortem data collection

The left and right eyes of n = 381 broilers were enucleated and weighed. The right eye of six birds per condition was taken on the final day of depletion (45 days old) to be frozen for histological examination. The eyes were stored in Dulbecco's Phosphate Buffered Saline (DPBS) while being transported from Sutton Bonington Campus to the Ophthalmology and Visual Sciences Department at the Queens Medical School where they were analysed. A section of the centre of the cornea was taken using a 7mm trephine and suspended in foil cups of optimum cutting temperature compound (OCT). The foil cups containing the suspended cornea section were then frozen with liquid nitrogen and stored at -80°C for frozen sectioning and histological analysis to confirm that the levels of UV exposure used in this study had not caused any damage to the cornea.

3.2.b) Dual Energy X-ray Absorptiometry (DEXA)

A sample of right broiler chicken legs (n = 278) was scanned in the frozen state using the Norland, Cooper Surgical Company, Fort Atkinson, WI XR-800™ DXA bone densitometer (serial no. 8598,). Daily calibrations were performed each day with the DXA machine initially scanning the Norland calibration standard (Serial no. 6560) followed by six quality control scans of the Norland phantom block (Serial no. 8498).

Small subject scans at a resolution of 1.0 x 1.0 were performed on a 250mm x 250mm user defined section of the total scanning area, which was sufficient to scan the whole legs of birds across all ages. All scans were initiated from the proximal end of the leg, which were orientated consistently with the medial side of the leg facing the scanner and the lateral side of the leg against the scanning bed. The *M. semimembranosus* in the leg was marked as the bone-less baseline area. When the scan was completed the 8-sided drawing tool was used to mark an area around the whole leg, femur, tibia and metatarsal & phalanges as shown in figure 3.2.b. This was used to obtain values of BMD (g/cm^2) in addition to bone (g), lean (g), and fat(g) content of the area (cm^2) specified.
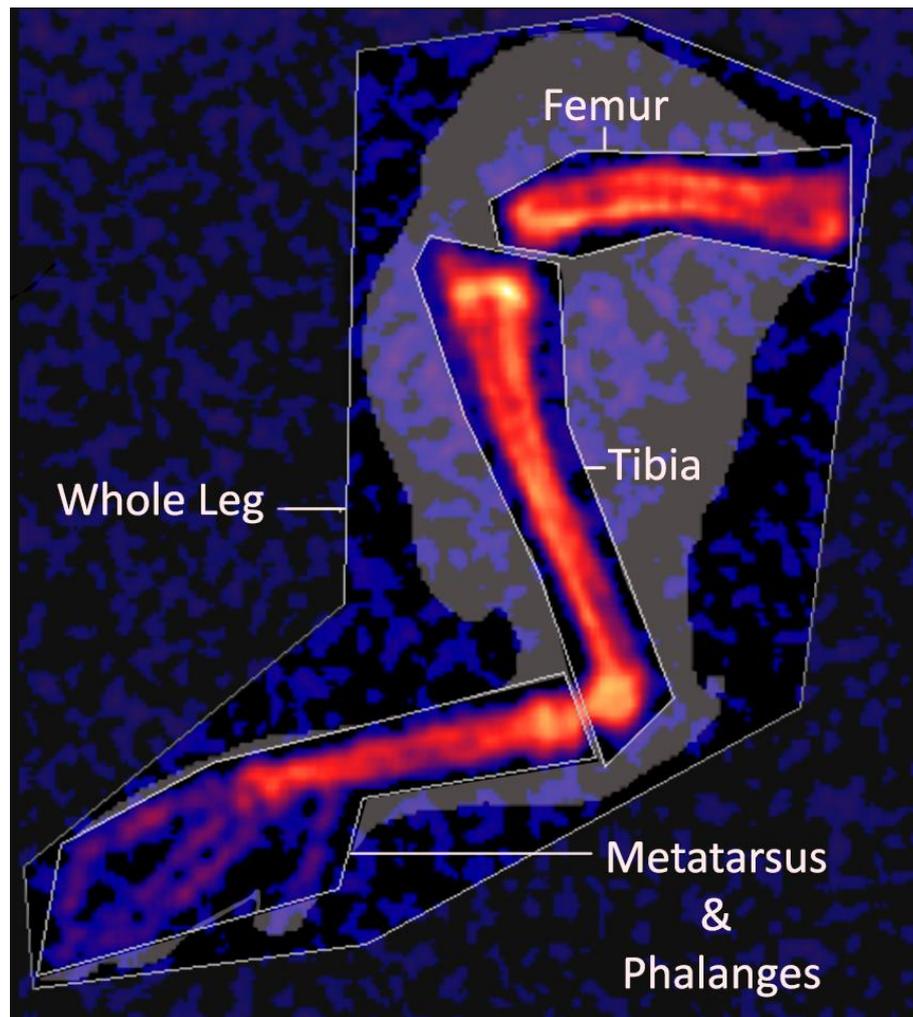
Figure 3.2.b - Orientation of legs during Dual Energy X-ray Absorptiometry (DEXA) and selection of leg segments for analysis of bone mineral density and leg composition.
The eight-point drawing tool was used to mark areas around the whole leg, femur, tibia and metatarsus & phalanges after scan measures of BMD. Bone (highest density) is shown in orange. The DEXA machine also measures lean and fat composition of the leg, as indicated by the grey overlay in this scan.

Samples scanned between 2 September 2015 and 12 December 2015 (n = 86) could not be included in the final analysis due to a fault with the DEXA scanner. While the scanner was correctly estimating bone mineral density based on measurements of the QC phantom, the estimates for lean and fat were incorrect, resulting in soft tissue being analysed as bone by the scanner. The scanner was repaired 19 September 2016 and only samples scanned after this date (n= 193, 64-65 per treatment) could be included in data analysis.

General linear models (glm) were constructed to investigate the impact of multiple independent variables (sex, flock, age and lighting treatment) and interactions between them on the dependent variable of bone mineral density (g/cm^2), bone content (g), fat content (g), and lean content (g) across the ages of 21, 30, 42, 43 and 44 days old in R statistical software (table 3.2.g). Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether a significant change in model fit (chi-squared test).

Pearson's product-moment correlations were performed (n = 48 tests, males and females analysed separately) across the whole data set on the following variables; end weight, drawing tool selected area (cm^2) of the whole leg, age, weight of the scanned (right) leg, bone mineral density (g/cm^2), bone content (g), fat content (g), and lean content (g) to eliminate multiple correlating independent variables from the models (to avoid multicollinearity), and to understand the association between the dependent variables and weight.

3.2.c) Bone Measurements

After DEXA imaging the legs were thawed overnight and dissected to obtain the lengths and diameters of the femur, tibia, metatarsus and third digit (phalange) using digital callipers. The foot was cut from the leg at the tibio-metatarsal joint to measure segment lengths (mm) of the third digit and metatarsus (fig 3.2.c.3-4). The femur and tibia were excised, and the lengths and diameters were measured (fig 3.2.c.1-2). The tibia of each bird was wrapped in a plastic bag and retained for texture analysis the same day.

Bone measurements statistical analysis

General linear models (glm) were constructed to investigate the impact of multiple independent variables (Sex, Flock, Age and Lighting treatment) and interactions between them on the dependent variables of bone measurements; including femur, tibia, metatarsus and third digit lengths (mm) and diameters (mm) across the ages of 9, 21, 30, 42, 43 and 44 days old in R statistical software (Table 3.2.g). Backwards elimination was used to exclude variables from GLMs, based on whether a significant change in model fit (chi-squared test).

Final models were selected using the flow chart detailed in the appendix (X.I). Pearson's product-moment correlations were performed (n = 54 tests, males and females analysed separately) across the whole data set on the following variables; end weight, Age, weight of the whole (right) leg, femur, tibia, metatarsus and third digit lengths (mm) and diameters (mm) to eliminate multiple correlating independent variables from the models (to avoid multicollinearity), and to investigate the association between the dependent variables and weight.
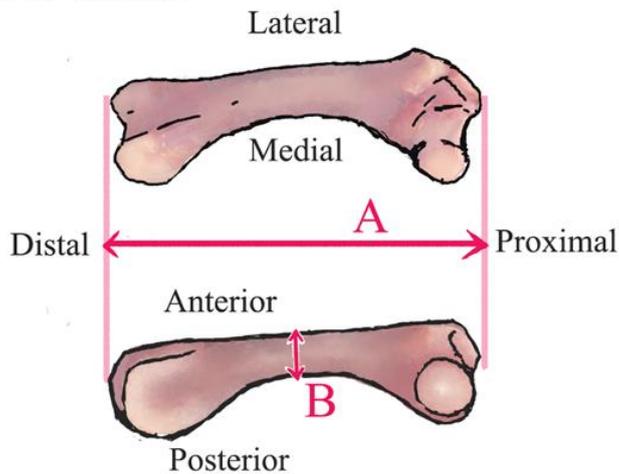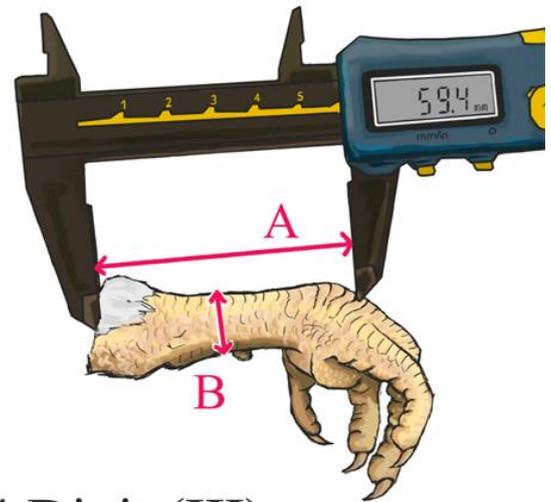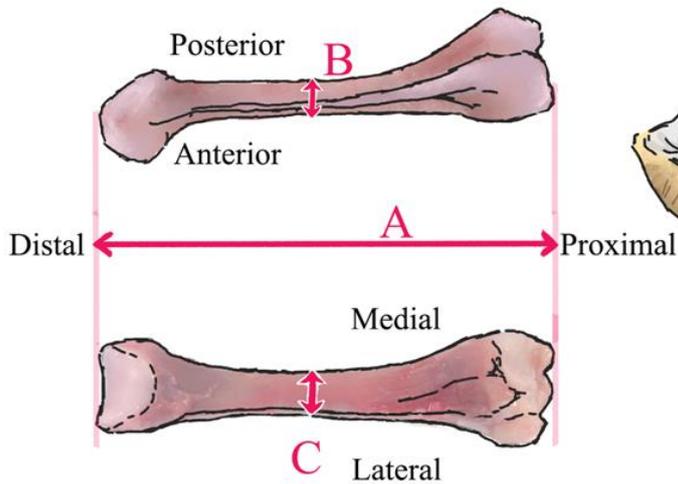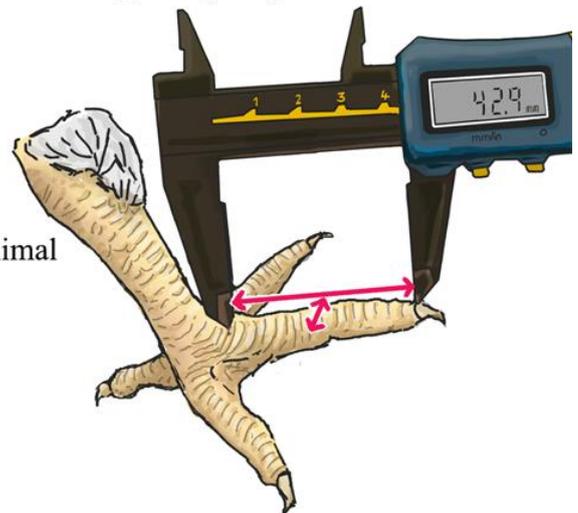
Figure 3.2.c- Orientation of bones and legs when taking segment lengths or bone measurements using digital callipers.

Femur (1) and tibia (2) diameter was measured across the antero-posterior axis (B). Tibia diameter was also measured across the mediolateral axis (C). Tarsometatarsus (3) length (A) and diameter (B) were measured with the digits flexed. Tarsometatarsus diameter was measured across the antero-posterior axis behind the metatarsal spur. digit III (4) length was measured with the foot in standing position from the base of the extended digit to the base of the claw. Digit diameter was measured across the top of the digit on the 2nd phalanx.

3.2.d) Tibia strength using texture analysis

Tibia were allowed to reach room temperature before breaking strength was measured using the Stable Micro Systems Texture Analyser (model: TA.HD.PLUS) with a factory calibrated 100kg load cell (Serial no.:11114713). The force and height were calibrated according to manufacturer instructions before each batch was tested using the Heavy Duty Platform (HDP/90 batch no.13502) and 3-point bending rig (HDP/3PB batch no. 13998). All tibia were consistently orientated across the two 6mm supports which were set at a constant distance of 38mm apart for all batches. The probe exerted force on the centre posterior surface of the tibia (figure 3.2.d) with measurements automatically taken at a trigger force of 50g, with a pre-test probe speed of 1.0 mm/sec, a test speed of 2.0mm/sec and a post-test speed of 10.00mm/sec. The peak force exerted on the bone was identified as the breaking force.
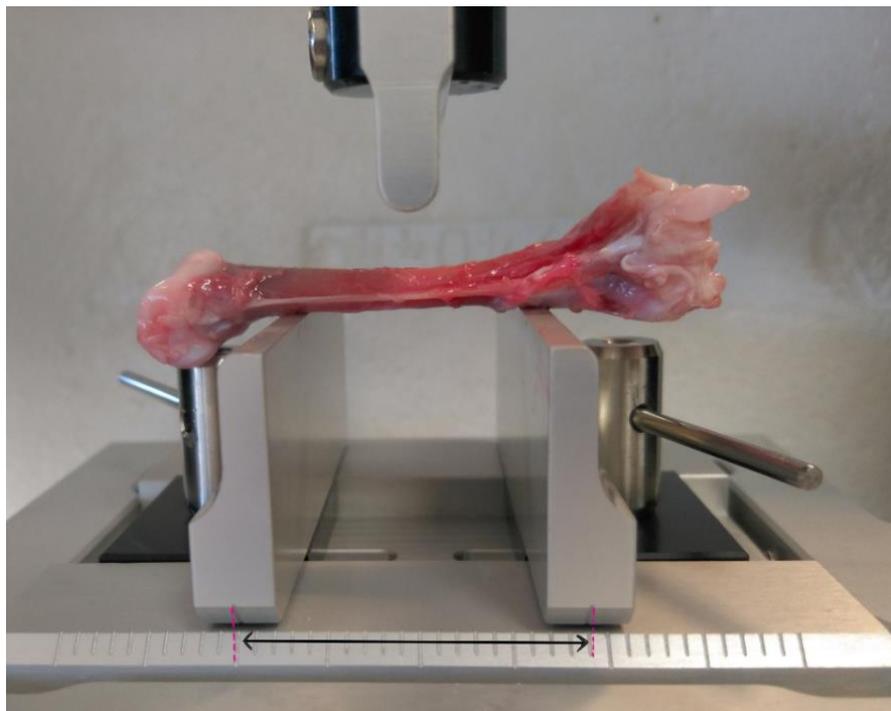
Fig 3.2.d- Three-point breaking tests to determine tibia breaking strength
Tibia were orientated across the supports 38mm apart (marked on the figure) so the centre of the probe applied force to the centre posterior surface of the tibia.

General linear models (glm) were constructed to investigate the impact of multiple independent variables (Sex, Flock, Age and Lighting treatment) and interactions between them on the dependent variable of tibia breaking strength (g) across the ages of 9, 21, 30, 42, 43 and 44 days old in R statistical software (Table 3.2.g). Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether a significant change in model fit (chi-squared test).

Pearson's product-moment correlations for males and females were performed across the whole data set to investigate associations between tibia breaking strength and weight.

3.2.e) Tibial Dyschondroplasia

Tibial dyschondroplasia (TD) is characterised by a mass of avascular cartilage in the metaphysis of the proximal ends of the tibia. Following texture analysis, the Proximal head of the tibia was cut with a scalpel to assess the presence and severity of TD and assigned a category (figure 3.2.e).

Figure 3.2.e – Representative images of the scoring system for severity of Tibial Dyschondroplasia (TD)
Images of broiler chicken tibia from the current experiments showing the severity of TD ranging from "None" to "Severe".

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Ordinal logistic regressions analysis (polr) was performed in R statistical software to investigate the effects of multiple independent variables (Sex, Age, flock, lighting treatment and interaction effects between these variables) on an ordered categorical dependent variable; "TD severity" (table 3.2.g). Final models were selected using the flow chart detailed in the appendix (X.I). Backwards elimination was used to exclude variables, based on whether a significant change in model fit (chi-squared test). Spearman's rank correlations were performed for males and females across the whole data set to explore the association between weight and TD.

3.2.f) Cornea Histology

Hematoxylin and eosin (H&E) and alcian blue stains

Corneas frozen in OCT compound and stored at -80°C as described in section x were cut into 7µm thick sections using a microtome cryostat (LEICA CM3050 S) and low profile microtome blades (LECIA 819). Nine sections were taken for each bird (three per slide), and allowed to dry on SuperFrost® microscope slides coated with 3-Aminopropyltriethoxysilane (APES), (Menzel Gläser) before being fixed in ice cold acetone (Sigma-Aldrich) for 30 seconds. One slide from each bird was stained with H&E and Alcian blue according to the protocols detailed in the appendix (X.II and x.III). Cover slides (Menzel Gläser) were applied with Distyrene Plasticizer Xylene (DPX) mounting medium (Fisher) And viewed with an upright Microscope (LEICA MC170 HD). Stained sections were thoroughly examined at a magnification of 20 and 40, and an image of the cornea was taken with a HD camera (LEICIA MC170) at a magnification of 40x using the LECIA Application Suite imaging software (v.4.8). The same microscope settings were maintained across all birds for H&E (exposure 30.ms, Gain 2.6 x, Saturation 151.0, Gamma 0.60) and alcian blue stains (exposure 30.ms, Gain 2.3 x, Saturation 129.0, Gamma 0.60).

These images were used to prepare a questionnaire where three researchers in Academic Ophthalmology were asked to assign the images of the cornea epithelium a score of: one (normal appearance), two (mild abnormality), or three (abnormal appearance). As the researchers did not primarily work with avian eyes the participants were made aware of which images showed birds from the control group but were unaware of which birds were assigned to the "UVA only" and "UVA and UVB" conditions.

For analysis scores of one (normal appearance) were entered as 0, scores of two (mild abnormality) were entered as 1 and scores of three (abnormal appearance) were entered as 2. Ordinal logistic regressions analysis (polr) was performed in R statistical software to investigate the effects of the independent variables "Rater" and " Treatment" on the dependent variable "cornea score". If no scores of ≥ 2 were recorded, then a generalised linear model (glm) with binomial family (analogous to a logistic regression) was used in place of polr. Inter-rater agreement for H&E and Alcian blue stains was determined using a Fleiss' Kappa test in R statistical software (Gamer, et al., 2012), and ordered logistic regression .

TUNEL assay for apoptotic cells

The Terminal deoxynucleotidyl transferase dUTP nick end labeling assay (TUNEL assay) is an established method for detecting the DNA fragmentation which is characteristic of apoptosis. A TUNEL assay was performed on six birds (two from each condition) with a further two birds from the control group (room 4) used to prepare a positive and an unlabelled negative control for this assay. The TUNEL assay used was the TREVIGEN TACS® 2 TdT-Fluor In Situ Apoptosis Dectection Kit, Catalog #4812-30-K.

The assay was performed according to kit instructions, with the exception of the initial fixing method after frozen sectioning. The kit protocol recommends fixing tissue in 3.7% buffered formaldehyde for 10 mins at room temperature. In this study slides had been fixed with ice cold acetone. Slides were removed

from storage in the cold room and allowed to dry before being rehydrated by immersion in 100%, 95% and 70% ethanol for 5 mins each.

The labelling procedure and preparation of reagents was performed as detailed in kit instructions. Cytonin™ was selected for the first incubation stage of the labelling procedure, for a 60-minute incubation. A positive control was prepared from cornea sections from a control bird as detailed in kit instructions by incubation with TACS Nuclease™ solution for 60 mins in a humidity chamber. The aim of this stage was to generate DNA breaks in every cell and show the permeabilization and labelling reaction was successful. The magnesium cation was selected to prepare the Labelling Reaction Mix. The TdT Enzyme was omitted from the labelling reaction mix to prepare an unlabelled negative control from cornea sections of a control bird to indicate the amount of background labelling associated with non-specific binding of Strep-Fluor. Prior to labelling with Strep-Fluor all slides were counterstained with a 0.5mg/ml 4',6-diamidino-2-phenylindole (DAPI) solution (Life Technologies) for 10 minutes.

Staining was viewed using an upright fluorescence microscope (BX51; Olympus, Southend-on-Sea, UK) and images were captured at 453 nm (DAPI) and 518nm (Strep-Fluor) with a black-and white camera (XM-10; Olympus) and coloured filters applied using Cell^F software (Olympus).

Table 3.2.g) Summary of main independent and dependent variables of interest,

Independent variables and interactions between them were included in generalised linear (n = 40 models; 20 for males and 20 for females) or ordinal logistic regression (Tibial dyschondroplasia only; n = 1 model for males and 1 for females) to determine their effects on the health indicators of interest; Bone mineral density and bone, fat and lean weight composition of the right leg, bone measurements, tibia breaking strength and tibial dyschondroplasia. Cornea histology was assessed through a questionnaire in which research ophthalmologists scored images as normal (1), mildly abnormal (2) or abnormal (3).

Independent variables	Dependent variables	
Sex	Bone mineral density (whole leg)	Femur diameter
Flock	Bone mineral density (femur)	tibia length
Lighting treatment	Bone mineral density (tibia)	tibia diameter 1 (anterior-posterior)
Age	Bone mineral density (tarsometatarsus and foot)	tibia diameter 2 (medial - lateral)
	Bone mineral content (whole leg)	tarsometatarsus length
	Bone mineral content (femur)	tarsometatarsus diameter
	Bone mineral content (tibia)	digit III length
	Bone mineral content (tarsometatarsus and foot)	digit III diameter
	Fat content of leg	Tibial dyschondroplasia score
	Lean muscle content of leg	Tibia breaking strength
		Cornea score

3.2.h) Corrections for multiple testing

Corrections for multiple testing were performed for all above models using a modified Bonferroni procedure (Haccou, et al., 1992). Models were ordered by p value from lowest to highest. P values were then adjusted by multiplying all p values in each model by the total number of models (n = 42) for the first model, and then subsequently multiplying by n - 1 (for the model with the second lowest p values) followed by n- 2, then n- 3, until the p values of the model with the highest p values were multiplied by n-41 and thus stayed the same. Unless otherwise stated, corrected p values are presented within text and figures.

3.3) RESULTS

3.3.a) Dual Energy X-ray Absorptiometry (DEXA)

To exclude correlating factors from models, Pearson's product moment correlations between variables were examined (Table 3.3.a.i). As a result, age was included in the models but not end weight (g), leg weight (g) or scan area (cm^2). Males and females (n = 99 males and 102 females, table 2.3.a.ii) were modelled separately due to differences in skeletal development. Log values of Bone mineral density (BMD g/cm^2), Bone content (BMC g), Lean weight (g) and Fat content (g) were used for analysis (general linear models).

Table 3.3.a.ii)- Numbers of male and female broiler chickens included in Dual Energy X-ray Absorptiometry (DEXA) analysis. There were 193 broiler chickens in total across each lighting treatment and age group. Table shows the sample sizes remaining after removal of all scans obtained before the 19th September 2016 from the data set, which were affected by a fault with the DEXA scanner.

n	Males			Females		
Age	Control	UVA	UVA+UVB	Control	UVA	UVA+UVB
21	9	2	9	3	10	3
30	10	0	9	2	12	3
42	3	0	3	4	5	1
43	16	0	15	4	20	5
44	11	0	12	6	19	5

3.3.a.i) Bone Mineral Density (BMD)

Before corrections for multiple testing, whole leg BMD was significantly increased in males in the UVA + UVB treatment (glm: df = 92, t = 2.026, p = 0.046) compared to control broilers (figure 3.3.iii). Analysis of the separate parts of the leg showed increased BMD in the metatarsus and foot (glm: df = 92, t = 2.134, p = 0.036), and a trend for increased BMD in the tibia (glm: df = 92, t = 1.699, p = 0.093), compared to control males. However, after corrections for multiple testing these results were non- significant (table 3.3.f).

There was no effect of lighting treatment on the BMD of female broiler chickens.

Whole leg BMD increased significantly with age for male (glm: df = 92, t = 22.654, p < 0.001), and female (glm: df = 97, t = 19.313, p < 0.001) broiler chickens.

Table 3.3.a.i – Pearson's product moment correlations for variables related to DEXA imaging

Associations are between variables related to DEXA imaging are shown for male (blue) and female (orange) broiler chickens across all treatment conditions. (significance for all correlations p < 0.001, correlations were not corrected for multiple testing).

Pearson's product-moment correlations	AREA (cm²)	END WEIGHT (g)	AGE (days)	RIGHT LEG WEIGHT (g)	BONE MINERAL DENSITY (g/cm²)	BONE CONTENT (g)	LEAN CONENT (g)	FAT CONTENT (g)
AREA (cm²)		Cor 0.967, t = 37.048, df = 96, 95%CI: 0.951 - 0.978	Cor 0.977, t = 44.579, df = 96, 95%CI: 0.965-0.984	Cor 0.965, t = 35.916, df = 96, 95%CI: 0.948-0.976	Cor 0.895, t = 19.657, df = 96, 95%CI: 0.847-0.929	Cor 0.970, t = 39.097, df = 96, 95%CI: 0.955-0.980	Cor 0.962, t = 35.695, df = 96, 95%CI: 0.944-0.975	Cor 0.547, t = 6.403, df = 96, 95%CI: 0.391-0.672
END WEIGHT (g)	Cor 0.968, t = 36.998, df = 91, 95%CI: 0.953 - 0.979		Cor 0.904, t = 34.973, df = 274 95%CI: 0.880 - 0.923	Cor 0.976, t = 58.809, df = 171, 95%CI: 0.968-0.982	Cor 0.936, t = 26.051, df = 96, 95%CI: 0.906-0.957	Cor 0.977, t = 44.775, df = 96, 95%CI: 0.966-0.984	Cor 0.985, t = 55.582, df = 96, 95%CI: 0.977-0.989	Cor 0.570, t = 6.789, df = 96, 95%CI: 0.418-0.690
AGE (days)	Cor 0.970, t = 37.938, df = 91, 95%CI: 0.955-0.980	Cor 0.913, t = 36.450, df = 266, 95%CI: 0.890- 0.931		Cor 0.962, t = 48.243, df = 188, 95%CI: 0.950- 0.9713	Cor 0.878, t = 17.972, df = 96, 95%CI: 0.823-0.917	Cor 0.947, t = 28.742, df = 96, 95%CI: 0.921-0.964	Cor 0.952, t = 30.489, df = 96, 95%CI: 0.929-0.968	Cor 0.473, t = 5.255, df = 96, 95%CI: 0.303-0.614
RIGHT LEG WEIGHT (g)	Cor 0.959, t = 32.156, df = 91, 95%CI: 0.938- 0.972	Cor 0.978, t = 61.664, df = 169, 95%CI: 0.971-0.984	Cor 0.970, t = 54.396, df = 188, 95%CI: 0.960-0.977		Cor 0.938, t = 26.551, df = 96, 95%CI: 0.909-0.958	Cor 0.977, t = 44.709, df = 96, 95%CI: 0.966-0.984	Cor 0.995, t = 97.782, df = 96, 95%CI: 0.992-0.997	Cor 0.564, t = 6.696, df = 96, 95%CI: 0.412-0.686

Table 3.3.a.i. continued…

Pearson's product-moment correlations	AREA (cm²)	END WEIGHT (g)	AGE (days)	RIGHT LEG WEIGHT (g)	BONE MINERAL DENSITY (g/cm²)	BONE CONTENT (g)	LEAN CONENT (g)	FAT CONTENT (g)
BONE MINERAL DENSITY (g/cm²)	Cor 0.883, t = 17.980, df = 91, 95%CI: 0.829-0.913	Cor 0.968, t = 36.999, df = 91, 95%CI: 0.953-0.979	Cor 0.912, t = 21.21, df = 91, 95%CI: 0.870-0.941	Cor 0.927, t = 23.627, df = 91, 95%CI: 0.892-0.951		Cor 0.972, t = 40.158, df = 96, 95%CI: 0.958-0.981	Cor 0.929, t = 24.533, df = 96, 95%CI: 0.895-0.952	Cor 0.578, t = 6.945, df = 96, 95%CI: 0.429-0.697
BONE CONTENT (g)	Cor 0.970, t = 37.992, df = 91, 95%CI: 0.955-0.980	Cor 0.980, t = 47.033, df = 91, 95%CI: 0.970-0.987	Cor 0.963, t = 33.98, df = 91, 95%CI: 0.944-0.975	Cor 0.972, t = 39.291, df = 91, 95%CI: 0.958-0.981	Cor 0.972, t = 40.158, df = 91, 95%CI: 0.958-0.981		Cor 0.971, t = 39.989, df = 96, 95%CI: 0.957-0.981	Cor 0.568, t = 6.769, df = 96, 95%CI: 0.417-0.689
LEAN CONENT (g)	Cor 0.953, t = 30.068, df = 91, 95%CI: 0.930-0.969	Cor 0.981, t = 48.059, df = 91, 95%CI: 0.971-0.987	Cor 0.964, t = 34.801, df = 91, 95%CI: 0.964-0.976	Cor 0.992, t = 77.578, df = 91, 95%CI: 0.989-0.995	Cor 0.926, t = 23.389, df = 91, 95%CI: 0.890-0.950	Cor 0.969, t = 37.249, df = 91, 95%CI: 0.953-0.979		Cor 0.505, t = 5.737, df = 96, 95%CI: 0.341-0.640
FAT CONTENT (g)	Cor 0.664, t = 8.460, df = 91, 95%CI: 0.532-0.764	Cor 0.654, t = 8.251, df = 91, 95%CI: 0.520-0.654	Cor 0.632, t = 7.771, df = 91, 95%CI: 0.491-0.740	Cor 0.648, t = 8.114, df = 91, 95%CI: 0.512-0.752	Cor 0.626, t = 7.661, df = 91, 95%CI: 0.484-0.736	Cor 0.643, t = 8.017, df = 91, 95%CI: 0.506-0.749	Cor 0.615, t = 7.440, df = 91, 95%CI: 0.470-0.728	
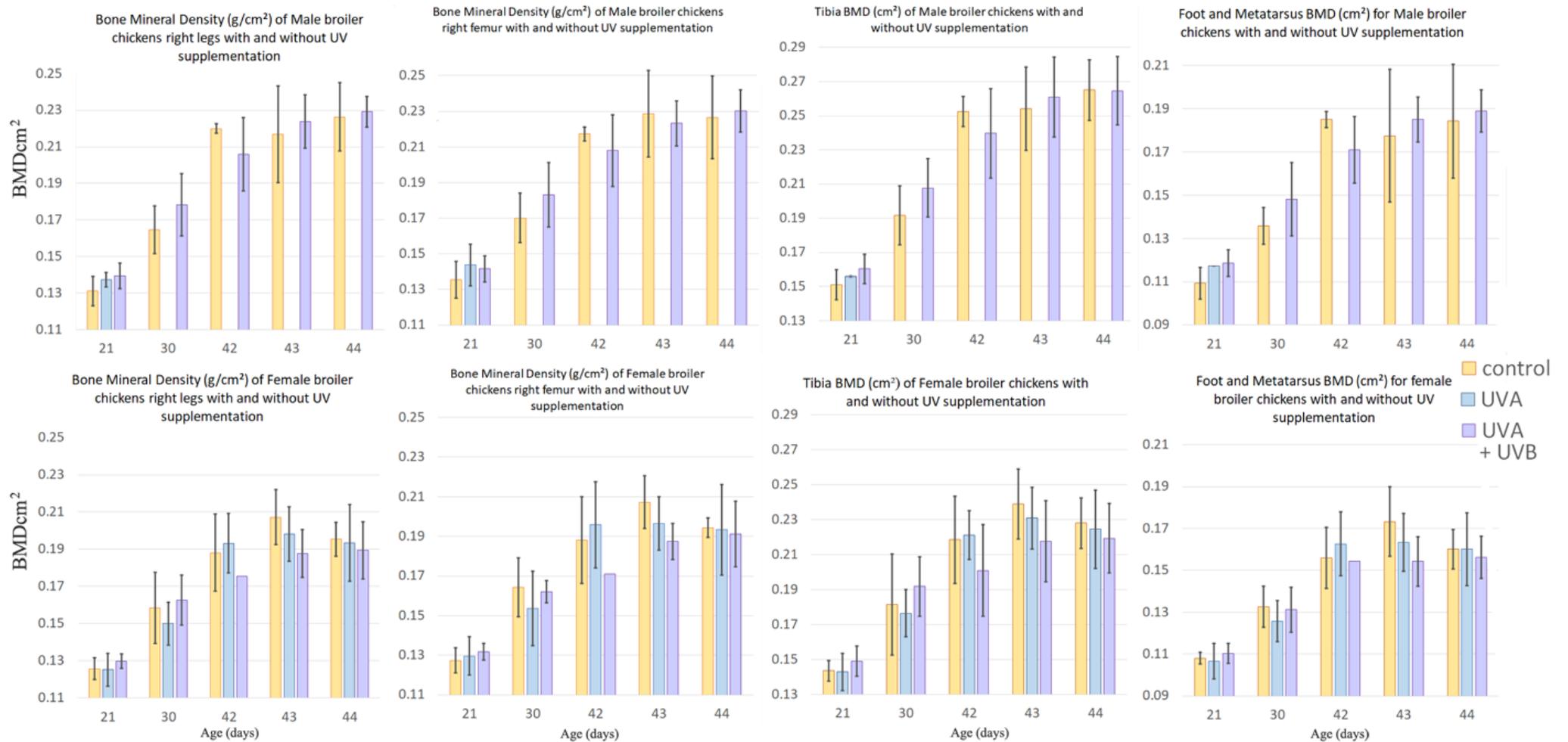
figure 3.3.a.iii)- Mean bone mineral density (g/cm²) of male and female broilers with and without UV wavelength supplementation

After corrections for multiple testing there was no effect of lighting treatment on the Bone mineral density (BMD) of Male or female broiler chickens (p >0.05). BMD increased with age for male and female broiler chickens (p < 0.001 for whole leg, femur, tibia and metatarsus and foot). A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

3.3.a.ii) Bone Mineral Content (BMC)

Before corrections for multiple testing, whole leg BMC was increased in males in the UVA + UVB treatment (glm: df = 92, t = 2.158, p = 0.034) compared to control broilers (figure 3.3.a.iv). Analysis of the separate parts of the leg showed increased BMC in the femur (glm: df = 92, t = 2.176, p = 0.032), tibia (glm: df = 92, t = 2.351, p = 0.021) and foot & metatarsus (glm: df = 92, t = 2.125, p = 0.036), compared to control males. After corrections for multiple testing none of these effects remained significant (table 3.3.f).

There was no effect of lighting treatment on the BMC of female broiler chickens.

Whole leg BMC increased with age for the whole leg, femur, tibia and foot and metatarsus of male (glm: df = 92, t = 42.657, p < 0.001), and female (glm: df = 97, t = 34.625, p < 0.001) broiler chickens.

3.3.a.iii) Lean content

Whole leg lean content (g) increased with age for male (glm: df = 92, t = 42.734, p < 0.001), and female (glm: df = 97, t = 36.16, p < 0.001) broiler chickens (table 3.3.a.v).

There was no effect of lighting treatment on the lean leg content (g) of male or female broiler chickens.

3.3.a.iv) Fat content

Whole leg fat content (g) increased with age for male (glm: df = 92, t = 7.456, p < 0.001), and female (glm: df = 97, t = 7.456, p < 0.001) broiler chickens (table 3.3.a.v).

There was no effect of lighting treatment on the leg fat content (g) of male or female broiler chickens.
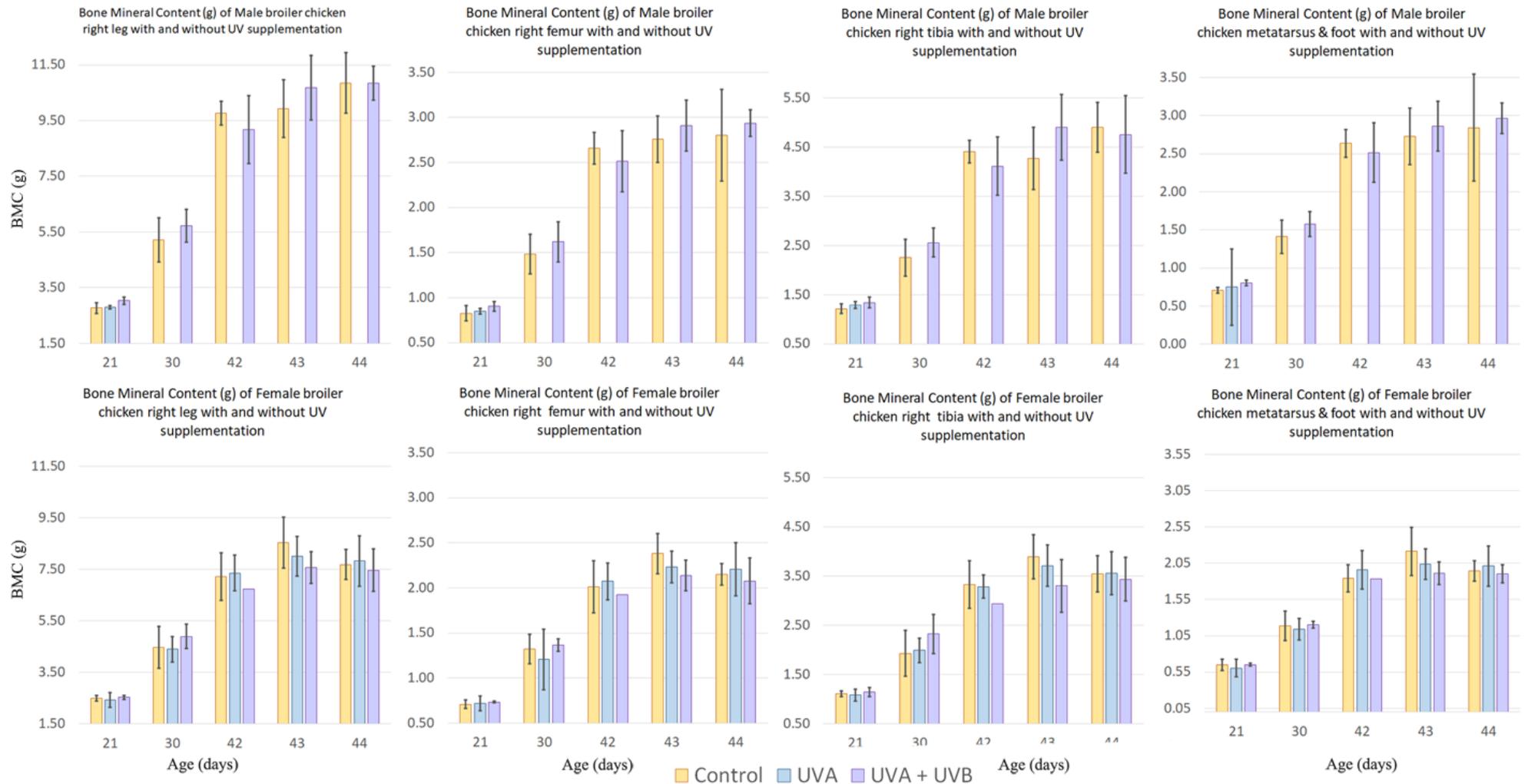
Figure 3.3.a.iv. mean bone mineral content (BMC) of broiler chicken legs with and without UV supplementation.
After corrections for multiple testing there was no effect of lighting treatment on the BMC of Male or female broiler chickens (p > 0.05). BMC increased with age for male and female broiler chickens (p < 0.001 for whole leg, femur, tibia and metatarsus and foot). A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

Table 3.3.a.v - lean (mean) and fat (mean) content (g) of male and female broilers whole legs with and without UV wavelength supplementation

There was no effect of lighting treatment on the lean and fat content of male or female broiler chickens ($p > 0.05$). Lean and fat content increased with age for male and female broiler chickens ($p < 0.001$). A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

Whole leg fat content (g)

Age	Control						UVA only						UVA + UVB					
(Days)	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD
21	3	12.89	4.83	9	13.85	3.19	10	10.16	2.96	2	18.05	2.81	3	14.10	1.20	9	14.33	3.52
30	2	26.41	4.00	10	29.33	7.03	12	22.25	3.27	0			3	24.96	4.00	9	30.77	7.03
42	4	23.42	7.46	3	29.64	6.57	5	28.54	8.26	0			1	21.72		3	29.39	12.48
43	4	34.62	9.64	16	35.30	7.88	20	26.83	6.98	0			5	23.81	6.23	15	36.80	8.12
44	6	15.17	7.26	11	30.20	3.85	19	22.02	6.19	0			5	18.91	3.73	12	28.12	8.19

Whole leg lean content (g)

Age	Control						UVA only						UVA + UVB					
(Days)	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD
21	3	87.79	5.89	9	98.14	10.39	10	83.18	11.22	2	111.90	2.55	3	84.80	7.39	9	111.28	8.25
30	2	154.60	44.12	10	186.54	16.92	12	156.63	11.40	0			3	163.80	22.56	9	196.14	26.05
42	4	253.83	32.07	3	330.90	13.67	5	251.60	13.04	0			1	266.30		3	307.22	27.48
43	4	292.08	31.27	16	330.85	31.00	20	277.33	29.33	0			5	254.58	14.65	15	348.49	31.96
44	6	281.83	12.37	11	382.19	31.14	19	275.14	36.29	0			5	275.44	20.89	12	377.88	30.32

3.3.b) Bone Measurements

To exclude correlating factors from models Pearson's product moment correlations between variables were examined (Table 3.3.b.i). As a result, age was included in the models but not end weight (g) or leg weight (g). Males and females were modelled separately due to differences in growth rates and skeletal development. Log values of bone measurements (mm) were used for analysis (generalised linear models) to improve model fit.

Femur length was not analysed as the femoral heads of some of the broiler chickens had been damaged when being dissected from the hip sockets. Before corrections for multiple testing the diameter of the tibia (posterior-anterior axis) was thicker in UVA + UVB treated males (glm: df = 134, t = 2.532, p = 0.013). This effect did not remain after corrections for multiple testing (Table 3.3.f). There were no other effects of lighting treatment on the bone or segment length measurements for male or female broilers (table 3.3.b.ii).

Bone measurements increased with age for males; (femur diameter glm: df = 129, t = 31.992, p < 0.001), (tibia length glm: df = 134, t = 48.539, p < 0.001), (tibia diameter posterior- anterior surface glm: df = 134, t = 31.363, p < 0.001), (tibia diameter medial -lateral surface glm: df =134, t =28.204, p < 0.001), (tarsometatarsus length glm: df 132, t = 43.702, p >0.001), (tarsometatarsus diameter glm: df = 132, t =23.451, p = <0.001), (digit length glm: df = 132, t = 33.732, p <0.001), (digit diameter glm: df = 132, t = 16.905, p = <0.001) and females (femur diameter glm: df = 140, t = 31.474, p < 0.001), (tibia length glm: df = 140, t = 54.825, p < 0.001), (tibia diameter posterior- anterior surface glm: df = 140, t = 31.700, p < 0.001), (tibia diameter medial -lateral surface glm: df =140, t =25.770, p < 0.001), (tarsometatarsus length glm: df 138, t = 46.986, p <0.001), (tarsometatarsus diameter glm: df = 138, t =24.145, p = <0.001), (digit length glm: df = 137, t = 31.207, p <0.001), (digit diameter glm: df = 138, t = 18.265, p = <0.001).

Table 3.3.b.i - Pearson's product moment correlations for variables related to female segment lengths and bone measurements

 The association between variables related to segment lengths and bone measurements for female broiler chickens was investigated across all treatments (significance for all correlations p < 0.001).

Pearson's product-moment correlations	END WEIGHT (g)	AGE (days)	RIGHT LEG WEIGHT (g)	Femur d	Tibia L	Tibia d1	Tibia d2	tarsomet L	tarsomet d	Digit (III) L	Digit (III) D
END WEIGHT (g)		Cor 0.904, t = 34.973, df = 274 95%CI: 0.880 - 0.923	Cor 0.976, t = 58.809, df = 171, 95%CI: 0.968-0.982	Cor 0.798, t = 14.650, df = 122, 95%CI: 0.724-0.854	Cor 0.950, t = 33.466, df = 122, 95%CI: 0.929-0.964	Cor 0.837, t = 16.86, df = 122, 95%CI: 0.774-0.883	Cor 0.765, t = 13.126, df = 122, 95%CI: 0.681-0.830	Cor 0.939, t = 29.845, df = 120, 95%CI: 0.913-0.957	Cor 0.784, t = 13.843, df = 120, 95%CI: 0.705-0.844	Cor 0.829, t = 16.163, df = 119, 95%CI: 0.763-0.878	Cor 0.534, t = 6.911, df = 120, 95%CI: 0.393-0.650
AGE (days)			Cor 0.962, t = 48.243, df = 188, 95%CI: 0.950- 0.9713	Cor 0.931, t = 30.101, df = 139, 95%CI: 0.905-0.950	Cor 0.989, t = 78.307, df = 139, 95%CI: 0.929-0.965	Cor 0.937, t = 31.492, df = 139, 95%CI: 0.912-0.954	Cor 0.900, t = 24.332, df = 139, 95%CI: 0.863-0.927	Cor 0.982, t = 60.026, df = 137, 95%CI: 0.974-0.987	Cor 0.911, t = 25.855, df = 137, 95%CI: 0.877-0.935	Cor 0.937, t = 31.422, df = 136, 95%CI: 0.914-0.955	Cor 0.825, t = 17.111, df = 137, 95%CI: 0.764-0.872
RIGHT LEG WEIGHT (g)				Cor 0.909, t = 25.762, df = 139, 95%CI: 0.876-0.934	Cor 0.969, t = 46.583, df = 139, 95%CI: 0.958-0.978	Cor 0.926, t = 28.985, df = 139, 95%CI: 0.899-0.955	Cor 0.903, t = 24.741, df = 139, 95%CI: 0.867-0.929	Cor 0.903, t = 24.742, df = 139, 95%CI: 0.867-0.929	Cor 0.961, t = 40.425, df = 137, 95%CI: 0.945-0.972	Cor 0.900, t = 24.107, df = 137, 95%CI: 0.862-0.927	Cor 0.921, t = 27.657, df = 137, 95%CI: 0.892-0.943

Table 3.3.b.i - Pearson's product moment correlations for variables related to male segment lengths and bone measurements

The association between variables related to segment lengths and bone measurements for male broiler chickens was investigated across all treatments (significance for all correlations p < 0.001).

Pearson's product-moment correlations	END WEIGHT (g)	AGE (days)	RIGHT LEG WEIGHT (g)	Femur d	Tibia L	Tibia d1	Tibia d2	tarsomet L	tarsomet d	Digit (III) L	Digit (III) D
END WEIGHT (g)		Cor 0.904, t = 34.973, df = 274 95%CI: 0.880 - 0.923	Cor 0.976, t = 58.809, df = 171, 95%CI: 0.968-0.982	Cor 0.889, t = 20.222, df = 109, 95%CI: 0.842-0.922	Cor 0.961, t = 37.271, df = 114, 95%CI: 0.945-0.973	Cor 0.832, t = 16.013, df = 114, 95%CI: 0.766-0.881	Cor 0.883, t = 20.129, df = 114, 95%CI: 0.835-0.918	Cor 0.943, t = 29.894, df = 112, 95%CI: 0.918-0.960	Cor 0.834, t = 15.98, df = 112, 95%CI: 0.768-0.882	Cor 0.849, t = 16.980, df = 112, 95%CI: 0.788-0.893	Cor 0.624, t = 8.451, df = 112, 95%CI: 0.497-0.725
AGE (days)			Cor 0.962, t = 48.243, df = 188, 95%CI: 0.950- 0.9713	Cor 0.955, t = 36.399, df = 128, 95%CI: 0.937-0.968	Cor 0.989, t = 78.359, df = 133, 95%CI: 0.945-0.974	Cor 0.932, t = 29.597, df = 133, 95%CI: 0.905-0.951	Cor 0.945, t = 33.308, df = 133, 95%CI: 0.923-0.961	Cor 0.985, t = 64.395, df = 131, 95%CI: 0.978-0.989	Cor 0.928, t = 28.596, df = 131, 95%CI: 0.900-0.949	Cor 0.951, t = 35.520, df = 131, 95%CI: 0.933-0.966	Cor 0.841, t = 17.777, df = 131, 95%CI: 0.783-0.884
RIGHT LEG WEIGHT (g)				Cor 0.933, t = 29.401, df = 128, 95%CI: 0.907-0.952	Cor 0.913, t = 25.933, df = 133, 95%CI: 0.881-0.938	Cor 0.914, t = 25.933, df = 133, 95%CI: 0.881-0.938	Cor 0.932, t = 29.751, df = 133, 95%CI: 0.906-0.951	Cor 0.963, t = 40.964, df = 131, 95%CI: 0.948-0.974	Cor 0.911, t = 25.206, df = 131, 95%CI: 0.876-0.936	Cor 0.929, t = 28.750, df = 131, 95%CI: 0.901-0.949	Cor 0.829, t = 16.975, df = 131, 95%CI: 0.767-0.876

Table 3.3.b.ii – Mean Bone and segment length measurements (mm) of male and female broiler chickens with and without UV supplementation
After corrections for multiple testing there were no effects of lighting treatment. All bone measurements significantly increased with age in males and females (p < 0.001). A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

Age (Days)	Control						UVA only						UVA + UVB					
	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD
Femur diameter (mm)																		
9	5	4.0	0.5	7	4.0	0.2	6	3.9	0.2	6	4.0	0.1	8	3.7	0.3	4	3.9	0.2
21	3	6.4	0.4	9	7.4	0.7	10	6.6	0.4	2	7.1	0.2	3	7.0	0.4	9	7.4	0.6
30	2	8.7	0.3	10	8.9	0.7	12	8.5	0.6	0			3	8.6	0.2	9	9.1	0.7
42	6	10.0	0.8	16	11.0	1.0	23	9.8	0.8	0			7	9.6	0.4	15	11.8	1.3
43	4	10.2	0.9	16	11.0	0.4	20	10.1	0.6	0			5	9.8	0.6	15	11.6	0.7
44	6	10.6	2.3	11	11.3	0.9	19	10.0	0.8	0			5	10.1	0.8	12	11.3	0.8
Tibia Length (mm)																		
9	5	50.2	2.8	7	50.9	1.3	6	49.5	1.3	6	51.3	0.6	8	50.5	1.4	4	50.8	1.8
21	3	79.5	2.5	9	80.9	2.0	10	77.1	2.5	2	80.2	0.6	3	77.5	1.2	9	82.6	1.3
30	2	93.1	4.5	10	97.7	4.6	12	95.2	3.7	0			3	97.0	2.9	9	100.4	2.9
42	6	115.3	4.7	16	121.8	3.5	23	115.8	3.8	0			7	114.4	3.4	15	119.4	5.4
43	4	119.1	3.4	16	121.6	3.2	20	117.2	3.0	0			5	116.6	2.7	15	124.2	2.7
44	6	115.9	2.7	11	125.1	3.4	19	117.2	2.6	0			5	117.1	1.8	12	124.0	2.2
Tibia diameter 1 (mm)																		
9	5	3.3	0.2	7	3.2	0.2	6	3.1	0.1	6	3.4	0.4	8	3.0	0.2	4	3.3	0.3
21	3	5.1	0.5	9	5.9	0.5	10	5.4	0.3	2	5.7	0.6	3	5.1	0.1	9	6.0	0.4
30	2	6.6	0.6	10	7.1	0.6	12	6.6	0.6	0			3	7.1	1.0	9	7.4	0.5
42	6	7.7	0.7	16	8.8	0.6	23	7.6	0.6	0			7	7.3	0.3	15	10.1	1.5
43	4	8.2	0.8	16	8.9	0.6	20	8.1	0.6	0			5	7.9	0.2	15	9.6	0.9
44	6	7.6	0.3	11	9.4	0.8	19	7.7	0.7	0			5	7.7	0.6	12	9.3	0.7

Table 3.3.b.ii continued…

Age	Control						UVA only						UVA + UVB					
(Days)	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD
Tibia diameter 2 (mm)																		
9	5	3.9	0.7	7	3.7	0.3	6	3.6	0.4	6	3.7	0.5	8	3.5	0.7	4	3.9	0.5
21	3	6.5	0.6	9	7.6	0.5	10	6.7	0.5	2	7.5	0.6	3	6.7	0.2	9	7.6	0.6
30	2	8.5	2.3	10	9.5	0.7	12	8.2	1.0	0			3	8.3	1.9	9	10.0	0.9
42	6	9.4	0.8	16	10.7	0.9	23	9.0	0.9	0			7	8.8	0.9	15	11.0	1.0
43	4	10.6	1.0	16	11.9	0.7	20	10.4	1.1	0			5	10.0	0.7	15	12.4	0.7
44	6	10.3	0.6	11	12.6	1.0	19	10.2	1.0	0			5	10.0	0.9	12	12.3	1.1
Tarsometatarsus length (mm)																		
9	5	34.4	1.7	7	36.5	1.5	6	35.2	1.6	6	37.4	2.0	8	36.3	1.0	4	36.0	2.1
21	3	55.5	2.3	9	57.6	1.3	10	54.3	1.9	2	58.3	0.7	3	54.8	1.6	9	58.7	1.5
30	2	65.7	3.4	10	69.5	3.5	12	66.2	2.5	0			3	67.1	4.0	9	71.1	1.2
42	6	79.7	2.8	16	85.6	3.5	23	78.2	4.0	0			7	79.7	4.0	15	85.2	3.3
43	4	81.7	2.8	16	85.1	3.2	20	80.2	2.5	0			5	78.9	1.4	15	85.7	3.0
44	6	79.7	1.4	11	89.3	3.8	19	80.7	2.3	0			5	79.8	1.8	12	87.0	2.6
Tarsometatarsus diameter (mm)																		
9	5	6.50	0.74	7	6.32857	0.3	6	6.0	0.5	6	6.6	0.7	8	6.1	0.6	4	6.5	0.4
21	3	11.23	0.40	9	12.05	0.7	10	11.1	0.8	2	12.8	0.8	3	11.0	0.5	9	12.5	0.8
30	2	13.50	1.56	10	15.1	0.7	12	13.5	0.7	0			3	13.1	0.4	9	14.9	0.9
42	6	13.67	0.83	16	15.9571	0.8	23	13.7	0.8	0			7	13.2	0.8	15	15.9	2.2
43	4	14.88	0.77	16	16.7538	0.5	20	15.0	0.9	0			5	15.0	0.8	15	17.4	1.0
44	6	15.13	0.69	11	18.35	0.6	19	15.2	1.2	0			5	15.0	0.4	12	18.3	1.0

Table 3.3.b.ii continued…

Age	Control						UVA only						UVA + UVB					
(Days)	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD	n	Females	SD	n	Males	SD
Digit III Length (mm)																		
9	5	29.9	3.3	7	29.0	2.4	6	28.6	1.7	6	30.9	2.2	8	29.0	2.5	4	28.5	1.9
21	3	41.2	1.2	9	42.7	1.5	10	41.1	1.7	2	41.9	0.4	3	39.6	1.7	9	43.0	0.8
30	2	45.1	0.4	10	49.6	2.3	12	47.2	2.5	0			3	46.5	2.5	9	49.5	2.1
42	6	52.4	3.2	16	63.0	4.2	23	55.0	3.9	0			7	54.1	3.6	15	60.4	4.9
43	4	53.3	2.1	16	57.5	2.6	20	52.5	2.3	0			5	51.3	1.8	15	57.3	1.7
44	6	52.0	2.0	11	60.3	2.7	19	53.8	2.0	0			5	52.2	3.4	12	57.6	1.6
Digit III diameter (mm)																		
9	5	3.7	0.5	7	4.0	0.4	6	3.9	0.5	6	4.0	0.9	8	3.5	0.3	4	4.1	0.6
21	3	7.0	0.7	9	7.5	0.7	10	6.4	0.8	2	8.2	0.3	3	7.0	0.2	9	7.7	0.8
30	2	8.8	0.6	10	9.4	0.6	12	8.5	0.7	0			3	8.4	0.8	9	9.2	0.8
42	6	8.3	1.1	16	8.4	0.8	23	7.8	1.1	0			7	7.6	0.9	15	9.2	1.5
43	4	9.2	0.9	16	10.5	0.9	20	9.2	0.8	0			5	8.7	0.7	15	10.3	1.0
44	6	9.1	0.8	11	11.1	0.8	19	8.9	0.5	0			5	9.2	0.7	12	10.6	1.1

3.3.c) Tibia strength using texture analysis

Tibia strength was positively correlated with weight in females (cor: r = 0.727 df = 121, p < 0.001) and males (cor: r = 0.760, df = 117, p < 0.001). Due to differences in males and female growth curves the sexes were modelled separately. Log values of breaking force (g) were used for statistical analysis.

Tibia strength significantly increased with increasing age in female (glm: df = 137, t = 21. 563, p < 0.001) and male (glm: df = 140, t = 23. 573, p < 0.001) broilers (table 3.3.c). There was no effect of lighting treatment or flock on the tibia breaking strength in male or female broiler chickens.

Table 3.3.c – Mean Tibia breaking strength (kg) of male and female broiler chickens with and without UV supplementation.

Tibia strength significantly increased with age for male and female broilers (p <0.001). There was no impact of UV supplementation on tibia strength. A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

Age (Days)	Control					
	n	Females	SD	n	Males	SD
9	5	4.7	1.06	7	4.9	1.71
21	3	22.1	5.39	9	24.2	5.12
30	2	29.4	6.97	10	35.6	5.77
42	6	43.4	9.18	16	54.3	11.24
43	4	45.6	5.52	16	52.5	10.89
44	6	42.2	5.69	11	51.4	8.11
Age (Days)	UVA only					
	n	Females	SD	n	Males	SD
9	6	3.7	0.58	6	4.7	1.41
21	10	19.7	2.62	2	24.7	1.01
30	12	33.4	5.69	0		
42	23	41.9	8.22	0		
43	20	42.0	9.28	0		
44	19	41.9	9.57	0		
Age (Days)	UVA + UVB					
	n	Females	SD	n	Males	SD
9	8	3.7	0.85	4	4.5	0.78
21	3	21.0	1.45	9	27.2	4.51
30	3	37.7	14.80	9	44.3	4.54
42	7	42.2	9.77	15	46.3	10.04
43	5	40.0	6.39	15	52.9	8.93
44	5	38.3	9.92	12	49.8	7.96

3.3.d) Tibial Dyschondroplasia (TD)

The severity of Tibial Dyschondroplasia (TD) was not correlated with weight in females (sr: r = 0.191, n = 115, p = 0.040) or males (sr: r = -0.087, n = 111, p = 0.364). Males and females were modelled separately for ordinal logistic regressions (polr) analysis, due to uneven distribution of sexes across treatments in each flock.

There was a trend for severity of TD to increase with age (between the ages of 21-44 days old) in female broilers (polr: n = 115, t = 2.110, p = 0.07). There was no effect of age on the severity of TD in male broilers (between 21-44 days old). There was no effect of lighting treatment on the severity of TD in male or female broiler chickens (figure 3.3.d)

Figure 3.3.d) – Severity of Tibial Dyschondroplasia (TD) in male and female broiler chickens between 21-44 days old with UV wavelength supplementation. There was a trend for severity of TD to increase between the ages of 22-44 in female broilers (p = 0.07) but no effect of age in males (p = 0.174). There was no effect of lighting treatment on TD severity and no correlation with end weight for males (r = -0.087) or females (r = 0.191). A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

3.3.e) Ocular health

3.3.e.i) Eye weights

Due to significant differences in the growth curves of male and female broilers sexes were modelled separately. Log values of all weights were used for statistical analysis to improve model fit.

Eye weight (Table 2.3.e.i) significantly increased with age for males (glm: df= 189, t = 52.792, p < 0.001) and females (glm: df= 189, t = 45.433, p < 0.001). The eye weight of male and female broilers was not affected by lighting treatment.

Table 3.3.e.i – Mean eye weights of broiler chickens with UV supplementation
Mean eye weight (g) increased with age for males and females (p <0.001). There was no effect of lighting treatment on the eye weight. A low number of males randomly allocated to the UVA treatment resulted in no data being reported for males above 21 days old.

Age (Days)	Control					
	n	Females	SD	n	Males	SD
9	5	0.74	0.063	7	0.85	0.030
21	3	1.27	0.056	9	1.45	0.135
30	2	1.71	0.195	10	1.90	0.113
42	6	2.16	0.095	16	2.52	0.158
43	4	2.21	0.153	16	2.51	0.144
44	6	2.22	0.125	11	2.66	0.171

Age (Days)	UVA only					
	n	Females	SD	n	Males	SD
9	6	0.69	0.052	6	0.79	0.095
21	10	1.23	0.087	2	1.35	0.013
30	12	1.66	0.204	0		
42	23	2.07	0.152	0		
43	20	2.17	0.114	0		
44	19	2.17	0.103	0		

Age (Days)	UVA + UVB					
	n	Females	SD	n	Males	SD
9	8	0.80	0.082	4	0.78	0.076
21	3	1.20	0.033	9	1.43	0.108
30	3	1.64	0.066	9	1.98	0.169
42	7	2.11	0.196	15	2.58	0.174
43	5	2.23	0.123	15	2.61	0.161
44	5	2.08	0.143	12	2.57	0.167

3.3.e.ii) Cornea Histology

There were no notable effects of lighting treatment on cornea histology; including Hematoxylin and eosin (H&E), alcian blue and TUNEL stains.

One individual chicken was unable to be sectioned and another was omitted from the analysis due to feedback in the questionnaire that the section was not a good enough quality to assess the epithelium. This gave a final sample size of images from 6 control, 5 UVA and 5 UVA + UVB broilers for the questionnaire (figure 3.3.e.ii).

Mean scores for each treatment, based on the assigned scores of 1 (normal), 2 (mild abnormality), or 3 (abnormal epithelium) were: control 1.22, UVA 1.53, UVB 1.2 for the H&E staining and 1.06, 1.20, and 1.13 for Alcian blue staining. Interrater agreement determined by a Fleiss kappa test was 0.60, ($z = 4.76$, $p = <0.001$) for H&E stains and 0.62 ($z = 4.29$, $p = <0.001$) for Alcian blue stains which indicate good agreement between assigned scores. Ordered logistic regression analysis (H&E stains) or a generalised linear model (Alcian blue stain was analysed as binomial data as no scores of 3 were assigned by the respondents for these samples), revealed no significant effect of 'rater' or 'lighting treatment' on the cornea epithelial scores assigned for both stains.

Cornea epithelium stained for the fluorescent TUNEL assay were compared to positive and negative controls (Figure 3.3.e.iii). There was no difference in the number of apoptotic cells between treatments (Figure 3.3.e.iv).
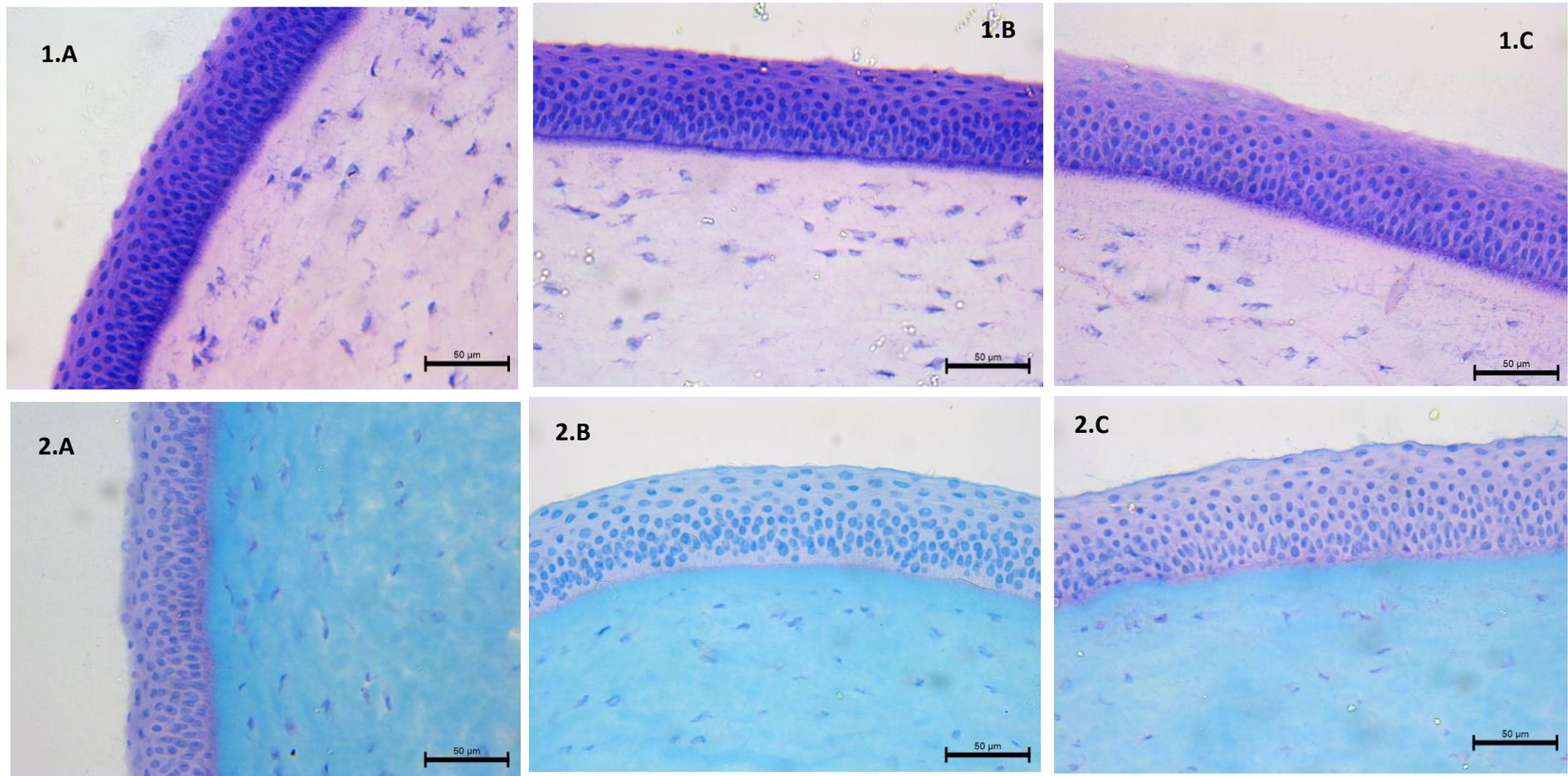
Figure 3.3.e.ii– Representative images of H&E (1) or alcian blue (2) stained cornea sections for (A) UVA, (B) UVA + UVB, and (C) control treatment broiler chickens at 40 X magnification. All examples shown were given a score of 1 (normal) by all three responding research ophthalmologists.
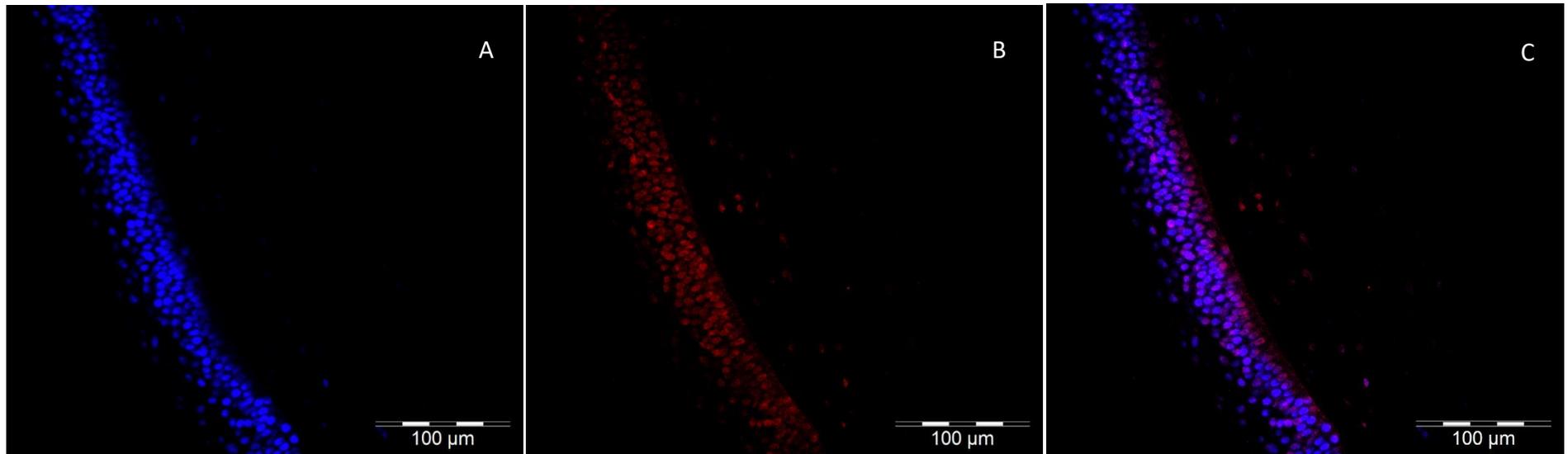
Figure 3.3.e.iii - TUNEL assay for Apoptosis positive control

Image A shows the DAPI counterstain of cell nuclei and B shows the Strep-Fluor binding for the positive control when incubated with TACS Nuclease™ solution. This generates DNA breaks in every cell to show the labelling reaction was successful. Image C is the combined output of the two images.
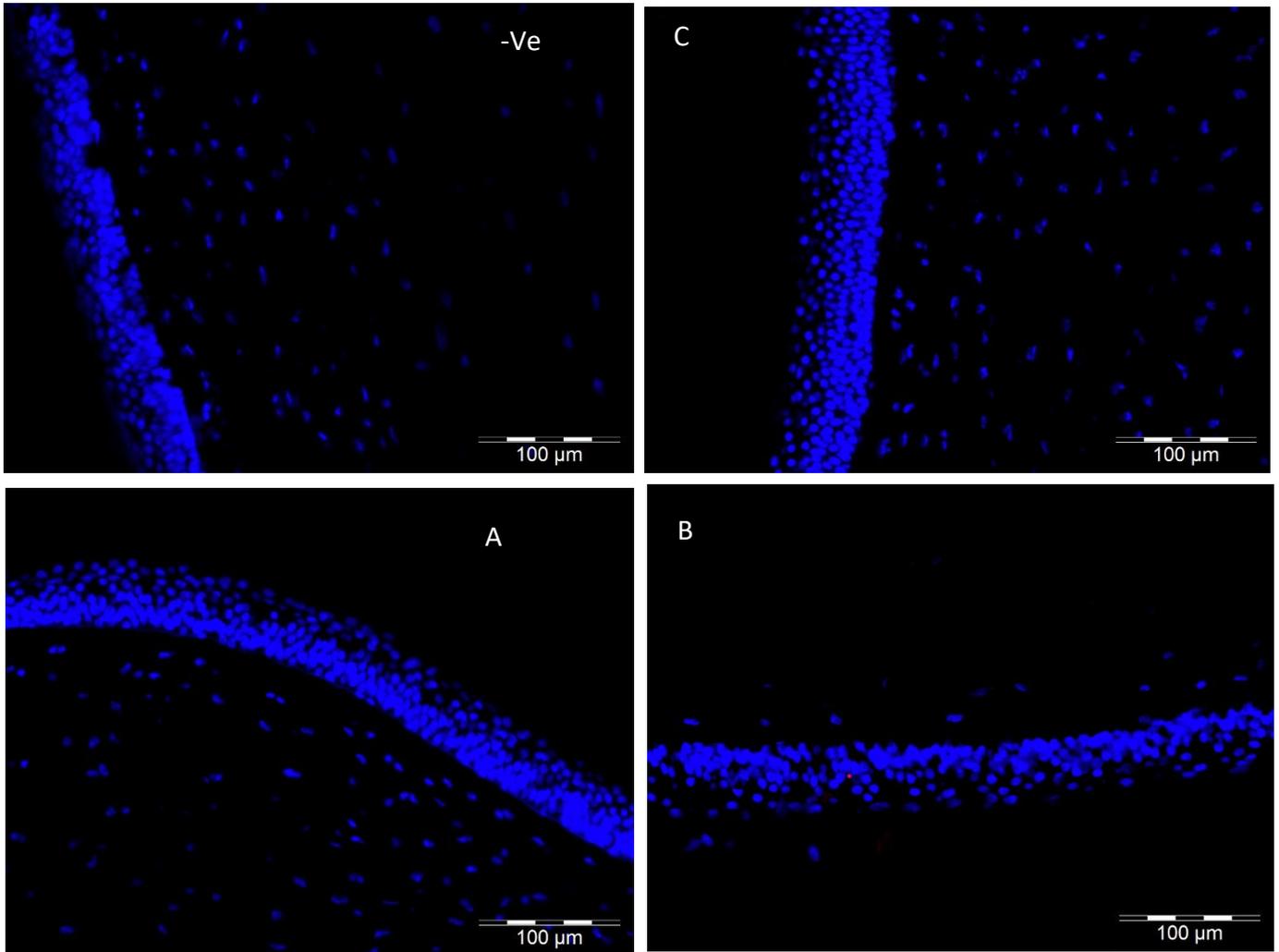
Figure 3.3.e.iv)- TUNEL assay for apoptosis

Images show combined signal output for DAPI (blue) and Strep-Fluor (Red) fluorescent labelling for the TUNEL assay for apoptosis. The unlabelled negative control (-ve) indicates the amount of background labelling associated with non-specific binding of Strep-Fluor. Labelled samples show no apoptotic cells in the UVA only (A), UVA+ UVB (B) or control treatment (C).

Table 3.3.f –The main impacts of ultraviolet wavelength exposure on broiler chicken health indicators

Estimates and SEM are shown for performance indicators of interest. Significant results (p < 0.05) from generalised linear models before corrections for multiple testing are italicised. Significant outcomes after adjustment of p values using the modified Bonferroni procedure (section 3.2.h) are emboldened

		Estimate	Standard Error	p	Adjusted p value
Bone mineral density (g/cm²)					
	MALES				
Whole leg	Control Vs UVA +UVB	0.015	0.007	*0.046*	0.229
	Age effect	0.009	< 0.001	*< 0.001*	**< 0.001**
Femur	Age effect	0.009	< 0.001	*< 0.001*	**< 0.001**
Tibia	Control Vs UVA +UVB	0.013	0.008	0.093	3.252
	Age effect	0.010	< 0.001	*< 0.001*	**< 0.001**
Metatarsus & foot	Control Vs UVA +UVB	0.021	0.010	*0.036*	0.249
	Age effect	0.009	0.001	*< 0.001*	**< 0.001**
	FEMALES				
Whole leg	Age effect	0.008	< 0.001	*< 0.001*	**< 0.001**
Femur	Age effect	0.008	< 0.001	*< 0.001*	**< 0.001**
Tibia	Age effect	0.009	< 0.001	*< 0.001*	**< 0.001**
Metatarsus & foot	Age effect	0.008	< 0.001	*< 0.001*	**< 0.001**
Bone content (g)					
	MALES				
Whole leg	Control Vs UVA +UVB	0.022	0.010	*0.034*	0.378
	Age effect	0.024	0.001	*< 0.001*	**< 0.001**
Femur	Control Vs UVA +UVB	0.025	0.011	*0.032*	0.322
	Age effect	0.022	0.001	*< 0.001*	**< 0.001**
Tibia	Control Vs UVA +UVB	0.031	0.013	*0.021*	0.188
	Age effect	0.024	0.001	*< 0.001*	**< 0.001**
Metatarsus & foot	Control Vs UVA +UVB	0.032	0.015	*0.036*	0.291
	Age effect	0.024	0.001	*< 0.001*	**< 0.001**
	FEMALES				
Whole leg	Age effect	0.021	0.001	*< 0.001*	**< 0.001**
Femur	Age effect	0.021	0.001	*< 0.001*	**< 0.001**
Tibia	Age effect	0.022	0.001	*< 0.001*	**< 0.001**
Metatarsus & foot	Age effect	0.022	0.001	*< 0.001*	**< 0.001**
Bone Measurments					
	MALES				
Tibia diameter	Control Vs UVA +UVB	0.024	0.009	*0.013*	0.075
(Anterior	Age effect	0.012	< 0.001	*< 0.001*	**< 0.001**
- posterior					

3.4) DISSCUSSION

The findings presented here (summarised in Table 3.4) suggest that the UV supplementation provided in the current study did not significantly impact skeletal health indicators and had no negative impacts on ocular health.

Table 3.4 – Summary of the impacts of UVA and UVB wavelengths in the current study,

There was a trend for males in the UVA + UVB treated group to have thicker tibias along the Anterior -posterior axis* There were no significant impacts observed due to UV supplementation on other measures after corrections for multiple testing.

	Impact summary	
health Indicator	UVA (18 hrs)	UVA + UVB (8 hours)
Bone Mineral Density	no impact	no impact
Bone Mineral Content	no impact	no impact
Bone measurements	no impact	Trend for increased male tibia thickness (Anterior - posterior axis)
Tibia breaking strength	no impact	no impact
Tibial Dyschondroplasia	no impact	no impact
Ocular health	no impact	no impact

3.4.a) Bone mineral density (BMD), bone mineral content (BMC), Bone measurements and Tibia Breaking Strength

The provision of UVA for the full 18-hour photoperiod, or a combination of UVA + UVB did not affect the BMD or BMC of broiler chickens in the current study after corrections for multiple testing.

While some differences in BMD and BMC were significantly improved for males in the UVA+UVB treatment before corrections for multiple testing, there were no corresponding impacts on tibia breaking strength, limb segment lengths or bone measurements, indicating the small increases were not associated with biologically relevant improvements in the mechanical strength of bone.

Female broiler chickens in the UVA only group also showed no improvements in the skeletal health indicators measured. Han *et al.* (2015) measured the femur, tibia and metatarsus of broiler chickens, and found the dry bone weight, diameter and ash weight of the three bones were higher in males compared to females of similar ages. These sex-based differences mean it is not possible draw conclusions on the effects of the UVA only treatment on males based on these results.

Many studies that demonstrate improved bone mineral density due to UVB exposure (Edwards et al. ,1994; Edwards, 2003) or dietry supplementation with vitamin D metabolites (Fritts & Waldroup, 2003; Ledwaba & Roberson, 2003; Whitehead, et al., 2004; Gómez-Verduzco, et al., 2013) use imbalenced or nutrient deficient experimental diets not representative of those used in commercial settings.

Therefore, while UVB provision may be more effective (Edwards, 2003; Tian, et al., 1994) and, due to the self-limiting nature of endogenous production, potentially safer (Nain, et al., 2007) than supplying dietary vitamin D, the high levels present in commericial broiler diets may limit the opportunity for further improvements to BMD, BMC and other indicators of skeletal health.

Alternatively, the lack of improvements in the skeletal health indicators observed may be due to limitations of the experimental set up. The current study allowed for broilers to avoid exposure to UVB using only a small "basking zone" , which would be achievable to implement in commerical practice and limits the risk of over-exposure to UV wavelengths. However, It is possible 8 hour provision of a small localised area of 30 μW/cm² UVB was not suffiecient to achieve improvements in the skeletal health measures in broilers kept at the current stocking density and group size.

3.4.b) Tibial Dyschondroplasia (TD)

The provision of UVA for the full 18-hour photoperiod, or a combination of UVA + UVB did not affect the severity of TD in broiler chickens in the current study. While a number of studies have found vitamin D supplementation (Fritts & Waldroup, 2003; Ledwaba & Roberson, 2003; Whitehead, et al., 2004; Gómez-Verduzco, et al., 2013) or UVB provision (Edwards et al. ,1994; Edwards, 2003) can reduce the incidence or severity of TD, other studies have noted no improvements (Elliot, 1992), or even increased levels of TD (Lofton & Soares, 1986) as a result of Vitamin D metabolite supplementation. Additionally, vitamin D metabolites were only effective at reducing TD in Ross cockrels when dietary calcium was low (Ledwaba & Roberson, 2003), so as the broilers in this study were fed a diet with adequate calcium this may have limited the potential for improvements.

However, a limitation of this study is the serum levels of Calcidiol were not measured. Therefore, it is also possible that the experimental set up did not allow birds to obtain sufficient UVB exposure to increase their vitamin D levels.

The exact pathogenicity of TD is unknown (Jahejo & Tian, 2020 in press), and the role of vitamin D in the development of TD unclear (Leach & Monsonego-Ornan, 2007). Variable experimental results may reflect the heritability of TD and genetic variation in modern lines of broilers (Mitchell, et al., 1997; Leach &

Monsonego-Ornan, 2007). While differences in susceptibility to TD have been found between slower growing and fast-growing strains (Shim, et al., 2012b), little difference has been found in TD prevalence between fast growing strains including Cobb and Ross strains commonly utilised in the UK (Dinev, et al., 2012; Kestin, et al., 1999).

The prevalence of TD lesions in Ross 308 flocks has been reported at 21 -24 % (Dinev, *et al.,* 2012; Nelson, *et al.,* 1992). Both of these studies scored only lesions (based on the size of the cartilage plug in the tibia) excluding mild disruptions to the growth plate. Applying these criteria to the current study would result in a prevalence of 17% in the broilers assessed for TD in the current study. The slightly lower prevalence in the current study could be due to a shorter photoperiod than that used by Dinev, *et al.,* (2012) as longer photoperiods have been associated with a higher prevalence of TD (Sorensen, et al., 1999), though an impact of amino acid supplementation but not photoperiod was found by (Petek, et al., 2005) indicating the multi-factorial nature of TD.

The expression of the Vitamin D receptor and its affinity for 1,25-(OH)2D3 was also found to be reduced in TD lesions (Berry, et al., 1996), indicating gene expression and receptor abundance may limit the potential of UVB supplementation to reduce the severity and incidence of TD. The potential role of gene expression is also highlighted by studies of broiler lines genetically selected for high and low incidences of TD (Mitchell, *et al.,* 1997), where both supplementation of Vitamin D metabolites or exposure to UVB lighting reduced TD in lines of broilers bred for low incidence of TD, but not those with high incidences, indicating differences in vitamin D metabolism between these lines (Mitchell, *et al.,* 1997). While this relates to selected populations, studies from a randomly mating fast growing broiler population also indicate TD is heritable (González-Cerón, *et al.,* 2015), which can contribute to variability in the extent environmental factors can impact the condition (Leach & Monsonego-Ornan, 2007).

Lines of broilers with different susceptibility to TD also do not differ significantly in body weight (Kuhlers & Daniel, 1996). This is in agreement with the current study, where no correlation was found between body weight and TD severity.

Therefore, UV lighting may not necessarily be a valid or cost-effective method for reducing TD in commercial settings. However, further investigations of the impacts of UV lighting and dietary vitamin D supplementation may be useful to determine which individuals may have compromised vitamin D metabolism and could aid the selection of more robust strains.

2.4.c) Conclusion

UVA provided for the whole 18-hour photoperiod or a combination of UVA + UVB for 8 hours of the photoperiod did not significantly impact skeletal health indicators measured in the current study and had no negative impacts on ocular health.

Further studies would be required in order to determine two main questions regarding UVB provision: 1) What UVB intensity is required for beneficial effects to bird health? and 2) How much of the floorspace must be covered by UVB to benefit bird health? In both cases, research should ideally be conducted on birds fed commercially relevant diets and kept at commercial stocking densities to determine if artificial supplementation of UVB wavelengths has further value in this context. Dietary vitamin D3 has a shorter half-life than endogenously produced vitamin D3 and is typically only 60% bioavailable bound to vitamin D–binding protein compared to 100% of endogenously produced vitamin D3 (Haddad & Chyu, 1971).

Therefore, while no improvements in skeletal health were observed in the current study, it should be noted that compromised skeletal health is an indicator of severe vitamin and mineral deficiencies and other aspects of health and could potentially still be improved even with dietary supplementation at recommended levels due to the shorter half-life of dietary D3 (Matos, 2008;

Williams, et al., 2010; Proszkowiec-Weglarz & Angel, 2013). Vitamin D supplementation has also been reported to improve other aspects of reproductive health and immune function (Gómez-Verduzco, et al., 2013; Saunders-Blades & Korver, 2015). Thus, further studies should expand on the range of health indicators measured, for example by examining humoral immune responses to vaccines used in commercial settings.

A limitation of the current study is that blood metabolites (calcium and phosphorus) and serum levels of Calcidiol were not measured, though this was attempted using a 25-Hydroxy Vitamin D assay (Diazyme Laboratories, Dresden, Germany), the assay could not be completed in time and serum samples (taken via cardiac puncture post-mortem) were often haemolysed to varying extents, which would greatly impact the results of any serological assays. A further limitation was the one of the measures of ocular health was a questionnaire with images of the H&E and Alcian blue stains of cornea sections scored by ophthalmologists. While interrater reliability was good, the questionnaire was not fully blinded as the control broilers were indicated. Providing a separate sheet with images of different sections of control birds and leaving the main questionnaire unlabelled would have been a better way to eliminate potential bias.

The next chapter will explore associations between selected performance, health and welfare indicators measured in the current study.

CHAPTER FOUR

4) THE IMPACT OF ULTRAVIOLET WAVELENGTHS ON BROILER CHICKEN PERFORMANCE, HEALTH AND WELFARE

4.1) INTRODUCTION

The links between good animal welfare, health and production are often complex; particularly under commercial settings where animal management is constrained by narrow profit margins.

Higher welfare standards, such as those imposed by certified organic farms in the UK, are reflected in the higher costs to consumers. However, the premium price also restricts these products to a smaller proportion of the consumer-base able to afford these higher welfare products, which may also come at the cost of being less sustainable (Niggli, 2015; Wachter, 2016; Wagenberg, et al., 2017).

Trade-offs between broiler welfare, health and production arise primarily as a consequence of selective breeding and modern husbandry practices that promote rapid growth (Bessei, 2006). Many metabolic disorders are strongly associated with modern production genotypes which are vastly different from earlier commercial lines or heritage breeds (Kestin, et al., 1992; Knowles, et al., 2008; Schmidt, et al., 2009; Kalmar, et al., 2013; Zuidhof, et al., 2014; Tallentire, et al., 2016).

Collectively, this evidence has led to the assumption that selection for high production traits coupled with modern farming methods are linked to inevitable and unavoidable adverse welfare consequences (European Commission, 2000; Thiruvenkadan, et al., 2011). However, multi-trait selection breeding programs with a focus on both performance and biological traits that improve bird welfare have been suggested as a potential solution for these issues (Dawkins & Layton, 2012).

Many suggested husbandry improvements, such as preventative strategies suggested to reduce leg disorders, would likely reduce overall outputs; including the use of slower growing broiler strains and other husbandry changes that limit growth (Knowles, et al., 2008). However, leg disorders themselves can also negatively impact productivity, resulting in reduced activity and birds spending increasing amount of time sitting down. If litter quality is poor this can lead to contact dermatitis, a condition which impacts both animal welfare, production, and can lead to carcass condemnation (Jong, et al., 2014).

The incidence of leg disorders and contact dermatitis have also been found to be worsened at higher stocking densities typical of commercial practice in a number of studies (Knowles, et al., 2008; Sanotra, et al., 2001; Shepherd & Fairchild, 2010; Farhadi, et al., 2016). However, higher stocking densities may not inherently cause welfare problems, but instead create greater challenges in maintaining environmental conditions within ideal parameters (Dawkins, et al., 2004).

Deviations from broiler chickens preferred environmental parameters, inadequate nutrition, poor water quality and disease can also activate stress and fear responses in broiler chickens (Maxwell, 1993; Lara & Rostagno, 2013). While stress hormones such as corticosteroids primarily have protective and adaptive effects; the intensity, duration, unpredictability and uncontrollability of a stressor may lead to deleterious effects and compromised welfare (Cockrem, 2013). Stress has significant economic consequences for the poultry industry; resulting from both the reduced growth and poorer feed efficiency of stressed animals and the morbidity and mortality associated with increased disease susceptibility (Lin & Decuypere, 2006; Lara & Rostagno, 2013; Gomes, et al., 2014; Calefi, et al., 2017), which may also pose a threat to public food safety (Humphrey, 2006).

The psychological impacts of stress are also an important welfare issue, as corticosterone administration has been shown to cause pessimistic judgement bias in broiler chickens (Iyasere, et al., 2017) and negatively impacts learning

abilities in broiler breeders (Buckley, et al., 2011). However, the link between stress and welfare is not always simple. Individual animals vary greatly in their susceptibility to stress-related pathologies under different contexts, making a "threshold" level of stress associated with compromised welfare challenging to identify (Cockrem, 2007; Pusch, et al., 2018).

Tickle, et al. (2018) found broiler chickens had a limited total energy budget, and resting metabolism accounted for an increasing proportion of this budget as birds developed, limiting the energy available for other activities and contributing to a decline in locomotor capacity. This may significantly compromise the welfare of broiler chickens, as they become increasingly less able to move and engage in normal behaviours, a problem that is often further exacerbated by lameness (Weeks, et al., 2000; Vestergaard & Sanotra, 1999).

However, the trajectory of declining activity with age in the study by Tickle, et al. (2018) was found to be less rapid in broilers raised in commercial settings compared to those raised in laboratory settings. This effect was attributed to differences in husbandry practices, including lighting regime. Previous studies have demonstrated that the distance travelled by broilers reared semi-commercially did not decrease at the end of the growth period (Aydin, 2016) and the activity of broiler chickens has been shown to be influenced by: light wavelength (Prayitno, et al., 1997a; Prayitno, et al., 1997b), light intensity (Kristensen, et al., 2006; Alvino, et al., 2009), photoperiod, (Calvet, et al., 2009) litter material and sequential feeding regimes (Bizeray, et al., 2002).

The research highlighted above demonstrates scope to limit the increasing metabolic costs of development on the welfare and behavioural expression of the broiler chickens through husbandry refinements and stress reduction. Research to explore and support evidence-based changes that benefit animal welfare is vital, and ideally proposed changes should be possible to implement on a commercial scale economically, limiting the cost impact to producers and consumers while benefiting as many animals as possible.

Further research investigating lighting regimes that promote good production, health and welfare have a clear potential to make an impact in this area. Lighting is already a legislated requirement for broiler chickens, but significant opportunities remain to refine commercial best-practice lighting regimes.

Furthermore, reliable indicators of performance, health and welfare are essential to monitor and assess the advantages and disadvantages of husbandry changes such as lighting manipulations.

It is therefore important to examine associations between different indicators, which often vary in their specificity and sensitivity under different contexts. If certain welfare indicators are found to be strongly correlated within individuals then fewer measures may need to be selected to assess welfare (Nicol, et al., 2011), which may help to limit unnecessary costs and time associated with assessing multiple measures (Botreau, et al., 2007; de Jong, *et al.,* 2016). The current chapter will explore the associations between some key performance, health and welfare indicators of interest (summarised in table 4.1).

Firstly, associations will be explored with the average daily gains between the ages of 8-15 days old. This time frame was chosen as it represents an early stage of growth and as data for both flocks was available between this time frame. Rapid growth is thought to be associated with a wide range of negative effects on broiler chicken welfare (Bessei, 2006), including; metabolic disorders (Julian, 1998; Kalmar, et al., 2013), incidence of tibial dyschondroplasia (Shim, et al., 2012b) and lameness (Kestin, et al., 2001; Paxton, et al., 2013).

The impacts of rapid growth on health and welfare outcomes has been investigated by comparing fast growing strains of broilers to slower growing strains (Dixon, 2020, Kestin, *et al*., 1992; Kestin, et al., 2001; Shim, et al., 2012; Shim, et al., 2012b; Rayner *et al.,* 2020) and by comparing the effects of ad-libitum and restricted diets (Acar, *et al.,* 1995; Corr, *et al.,* 2003). Faster growing broilers strains have larger, heavier bones which are denser and and stronger than lower growing strains (Shim, et al., 2012). However, faster growing broilers have been found to have poorer walking ability, higher mortality and

reduced expression of positive welfare indicators such as play, straw bale use and exploratory behaviours when compared to slower growing strains (Rayner *et al.,* 2020). Rayner *et al.* (2020) concluded that swapping to slower growing broiler genotypes would allow for the most welfare benefits.

However, the fast growing strain used by Rayner *et al.* (2020) was kept according to different commercial management protocols compared to the two slower growing strains; including a different diet, reduced hours of darkness and a different temperature profile throughout the growing period. Therefore, it is possible that modifying management procedures may facilitate welfare benefits even for more rapidly growing broilers. For example, walking speed and gait characteristics are improved when broilers are fed restricted diets (Corr*, et al.,* 2003). However, restricting feed intake is associated with other potential welfare implications if birds are hungry, leading to increased physiological stress and abnormal behaviours (Mench, 2002). Therefore lighting regimes that restrict feed intake to lesser extent or encourage activity may be a way to achive similar goals without the additional concerns of restricted diets (Alvino, et al., 2009a; Buyse, et al., 1996a; Olanrewaju, et al., 2006).

Secondly, the strength of association with gait score and other measures (tibia breaking strength, tibial dyschondroplasia, and tonic immobility time) will also be compared. Walking ability is often assessed using the Bristol gait score developed by Kestin, et al. (1992), or other simplified 3-point gait scoring systems in agreement with the Bristol Gait Score, but with the advantage of better within-observer and inter-observer reliability (Webster, et al., 2008; Garner, et al., 2010).

However, subjective measures of walking ability do not necessarily indicate the causes of lameness or the presence or absence of skeletal pathologies such as tibia curvature and tibial dyschondroplasia (TD), which may (Vestergaard & Sanotra, 1999) or may not (Garner, et al., 2010), be associated with gait score or other skeletal measures such as bone breaking strength (Kestin, et al., 1999; Sanotra, et al., 2001).

The associations between gait score and fear, as measured by tonic immobility duration, is also of interest as it is plausible that compromised mobility could cause chronic stress or fear. Broilers with TD have been found to have higher gait scores and longer tonic immobility times compared to broilers where TD was absent (Vestergaard & Sanotra, 1999; Sanotra, et al., 2001). Additionally, fear has also been measured using the avoidance- distance touch test included within the Welfare Quality® protocol (Bock & de Jong, 2010). This is a measure of human-animal relationship that assumes fearful broilers will withdraw from the observer. However, results of this test may be confounded by impairments in walking ability, not providing an accurate measure of fear of humans (Vasdal, *et al.,* 2018).

Finally, some previous studies have found associations between poor plumage condition and higher levels of fear in laying hens (Adams, et al., 1978; Lampang & Craig, 1990; Mahboub, et al., 2004). However, plumage condition was not associated with environmental choice in laying hens, which did not self-select environments where plumage was demonstrably improved or avoid environments where it was worse (Nicol, *et al.,* 2009). Less research has been conducted to examine these relationships in immature broiler chickens, particularly during the rapid phase of feather development as measured in the current study. Based on the literature reviewed above it was predicted that positive associations would be found with Average daily gains (ADG, g/day) between the ages of 8-15 with gait score, TD score and tibia breaking strength. Positive associations were also predicted with gait score and TI duration and TD score and TI duration. Possible associations were predicted where previous studies had found conflicting results to investigate these in the current study: gait score vs TD score, gait score vs tibia breaking strength, TD score vs tibia breaking strength, TI duration vs feather score and ADG, d/day between the ages of 8-15 with TI duration.

Table 4.1. Testing associations between performance health and welfare variables

Measures of performance (Average daily gains g/day between 8-15 days old), Health (Tibial dyschondroplasia score and tibial breaking strength) and welfare (Tonic immobility [TI] duration [mins], Gait score and feather score) were selected to examine associations of interest across individual broiler chickens. Predictions are indicated based on the findings of previous studies.

Variable 1	Variable 2	Hypothesis
ADG (g/day) 8-15 days old	Gait score	Positive association
ADG (g/day) 8-15 days old	TI time (mins)	Possible association
ADG (g/day) 8-15 days old	Tibial dyschondroplasia (TD score)	Positive association
ADG (g/day) 8-15 days old	tibia breaking strength (g)	Positive association
Gait score	Tibial dyschondroplasia (TD score)	Possible association
Gait score	TI time (mins)	Positive association
Gait score	tibia breaking strength (g)	Possible association
Tibial dyschondroplasia	tibia breaking strength (g)	Possible association
TI time (mins)	feather score	Possible association
TI time (mins)	Tibial dyschondroplasia (TD score)	Positive association

4.2) METHODS

4.2.a) Testing for associations between variables.

All analysis was performed in R statistical software (R Core Team, 2013). Pearson's product-moment correlations (n = 2) were performed to test for associations between continuous variables. Associations with ordinal measures were analysed using Spearman's rank correlations (n = 8). Both sexes and all treatments were analysed together to investigate associations across all broilers between the following variables:

1.   ADG (g/day) 8-15 days old   &   Gait score

2.   ADG (g/day) 8-15 days old   &   TI time (mins)

3.   ADG (g/day) 8-15 days old   &   Tibial dyschondroplasia (TD score)

4.   ADG (g/day) 8-15 days old   &   tibia breaking strength (g)

5.   Gait score   &   Tibial dyschondroplasia (TD score)

6.   Gait score   &   TI time (mins)

7.   Gait score   &   tibia breaking strength (g)

8.   Tibial dyschondroplasia   &   tibia breaking strength (g)

9.   TI time (mins)   &   feather score

10.   TI time (mins)   &   Tibial dyschondroplasia (TD score)

4.2.b) Corrections for multiple testing

Corrections for multiple testing were performed for all correlations using a modified Bonferroni procedure (Haccou, et al., 1992). Results were ordered by p value from lowest to highest. P values were then adjusted by multiplying all p values by the total number of tests performed (n = 10) for the first test (with the lowest p value), and then subsequently multiplying by n - 1 (for the test with the second lowest p value) followed by n- 2, then n- 3, until the test with the highest p value was multiplied by n-9 and thus stayed the same. Unless otherwise stated, corrected p values are presented within text and figures.

4.3) RESULTS

There was a weak positive correlation with ADG g/day between 8-15 days of age and gait score and a weaker correlation with ADG g/day between 8-15 days of age and tibia breaking strength (table 4.3). No other significant associations were found (Scatter plots for the selected associations are shown in Appendix X.IV).

Table 4.3. Association between selected performance health and welfare indicators for male and female broiler chickens.

Pearson's product moment (r) and spearman's rank (rs) correlations for all broiler chickens. Significant associations (p < 0.05) before corrections for multiple testing are italicised. Significant outcomes after adjustment of p values using the modified Bonferroni procedure (section 4.2.b) are emboldened.

Variable 1	Vs	Variable 2		correlation	df	p value	corrected p value
ADG (g/day) 8-15 days old		Gait score	rs	0.314	290	*<0.001*	**< 0.001**
ADG (g/day) 8-15 days old		TI time (mins)	r	0.072	299	0.213	1.491
ADG (g/day) 8-15 days old		Tibial dyschondroplasia (score)	rs	0.051	226	0.446	2.228
ADG (g/day) 8-15 days old		tibia breaking strength (g)	r	0.217	243	*<0.001*	**0.006**
Gait score		Tibial dyschondroplasia (score)	rs	-0.028	147	0.737	2.211
Gait score		TI time (mins)	rs	-0.024	260	0.695	2.779
Gait score		tibia breaking strength (g)	rs	0.182	162	*0.020*	0.158
Tibial dyschondroplasia (score)		tibia breaking strength (g)	rs	0.082	226	0.218	1.308
TI time (mins)		Tibial dyschondroplasia (score)	rs	-0.025	143	0.761	1.522
TI time (mins)		feather score	rs	0.007	294	0.908	0.908

4.4) DISSCUSSION

Weak positive correlations were found with ADG g/day between 8-15 days of age and gait score and a weaker correlation with ADG g/day between 8-15 days of age and tibia breaking strength (table 4.3). No other significant associations were found.

4.4.a) Associations with Average daily gains (g/day) between 8- 15 days of age

Higher average daily weight gain of broilers between 8-15 days (ADG) was associated with higher gait scores at 31 days old. This is consistent with previous findings showing a relationship between rapid growth and higher gait scores (Su, *et al.*, 1999; Kestin, *et al.,* 1991; Kestin, *et al.,* 2001; Sanotra, *et al.,* 2001; Kristensen, *et al.,* 2006). Higher ADG was positively associated with final tibia strength, which is in agreement with the general relationship that bone strength increases with weight and age (Williams, et al., 2004; Shim*, et al.,* 2012).

However, the relationship between ADG and tibia breaking strength was very weak, indicating a higher weight gain during this time was not strongly associated with higher bone strength. A potential explanation for this is the relatively poorer bone quality of fast-growing broiler chickens (Williams, et al., 2010). Slower growing strains of broiler chicken have been found to have improved bone quality in comparison to fast growing strains such as the Ross 308 broilers in the current study (Brickett, et al., 2007; Shim, et al., 2012). Feed restriction to limit rapid growth of fast-growing strains is also reported to improved bone mineralisation compared to those fed ad-libitum (Williams, et al., 2004), which would lead to comparatively weaker bones. However, feeding broilers lower energy diets to reduce their growth rate did not result in improvements in bone structure or composition in a previous study (Leterrier, et al., 1998).

The lack of a strong association between AGD and tibia strength may also be because tibia strength may be associated more with weight gain during a later phase of the growth after day 21, when the bone cortex became denser and mineralisation of cortical bone increases (Estefania, *et al.,* 2019; Williams, *et al.,* 2004).

There was no association found bettween ADG during an early growth stage and TI duration at 27 days of age. Fear related behaviours have been altered by the process of domestication, which alters the frequencies of behavioural patterns to favour less costly behaviour in breeds selected for high production (Price, 1984). Domesticed strains of chicken show reduced fear measures than their progentior species (Campler, *et al.,* 2009) and negative associations have been reported between fearfulness and production traits in broilers (Wang, et al., 2013; Duan, *et al.,* 2014), quail (Jones, *et al.,* 1997), and laying hens (Schütz, *et al.,* 2001).

In quail underlying fearfulness (including TI duration) was greater in birds genetically selected for low body weights compared to unselected controls and those selected for high body weights (Jones, *et al.,* 1997). Quanttitative Trait Locus analysis between fear-related behaviours and production traits in domestic laying hens, red jungle fowl and their progeny revealed phenotypic correlations bettween bettween production traits and fear-related reactions (Schütz, et al., 2004). This is consistent with resource allocation theory and indicates tradeoffs exist between resource-demanding production traits and energy-demanding behaviors such as fear related behaviours (Schütz, *et al.,* 2001; Schütz, et al., 2004).

Broiler chickens that obtain shorter TI durations have been found to have improved growth performance compared to those with long TI durations (Wang, et al., 2013). Administraion of corticosterone to mimic chronic stress in broiler chickens impairs muscle growth; with broilers exhibiting longer TI durations found to have depleated glycogen stores compares to those with shorter TI durations (Duan, *et al.,* 2014). Therefore, particularly under stressful conditions, a negative relationship bettween ADG and TI might be expected

which was not observed in the current study. The weight of broiler chickens at 27 days old (two days before the TI test) was also not a significant term when modelling TI duration or induction (section 2.3.b). A potential explaination for this could be the maximum TI duration used in the current study, which was 3 minutes, compared to a 5 minute (Nicol *et al.*, 2009) or 10 minute (Wang, et al., 2013; Schütz, *et al.,* 2001; Jones, *et al.,* 1997) used in other studies. Therefore it is not possible to rule out that an association may have been found if a higher TI duration limit had been used in the current study. However, expected average daily gains according to the Ross Performance Objectives during this time period at between 31-54 g (Aviagen, 2012), which all broilers in the current study met or exceeded, which could imply generally low levels of fear which did not impact production.

No association was found bettween ADG and TD score. TD has been found to be more prevalent in commerical fast growing strains compared to slow growing strains (Shim, et al., 2012b), and is thought to be a disorder associated with rapid growth (Leach & Monsonego-Ornan, 2007). However the underlying pathogenesis has not been fully determined (Jahejo & Tian, 2020 in press).

Weight bearing on the tibia was not found to be a primary factor in the development of TD (Riddell, 1975), which supports the findings in the current study. Lines of broilers selected for high and low susceptibility to TD were not found to differ significantly in body weight, suggesting TD expression and body weight were genetically and phenotypically independent traits (Kuhlers & Daniel, 1996). Additionally, Yalçin, et al., (2000) selected for a high or low incidence of TD for seven generations in broilers and found the low incidence strain had increased processing yields, indicating selection for TD would not impair final body weights and carcass yields. This is supported by the results of the current study, where no correlations were found with final body weights and TD scores (section 3.3.d), and has positive implications for the poultry industry, as selection against TD may   be possible without compromising performance.

4.4.b) Associations with gait score and Tibial dyschondroplasia

Previous studies have found Tibial Dyschondroplasia (TD) can lead to altered gait and higher gait scores (Julian, 1998; Farquharson & Jefferies, 2000; Sanotra, et al., 2001). However, no correlation between TD severity and gait score was observed in the current study, which is in agreement with results of Garner, et al. (2010) and Fernandes, et al., (2012). This implies higher (worse) gait scores and lameness may be more strongly influenced by factors other than TD such as valgus-varus angular bone deformitys (Fernandes, et al., 2012).

Associations between TI duration, gait score and severity of TD have also been reported (Vestergaard & Sanotra, 1999; Sanotra, *et al.,*2001). Suggesting broiler chickens with impaired mobility and a reduced capacity to respond to threats may have higher fear levels and longer tonic immobility (TI) durations.

However, the current study found no correlations between gait score, TD severity and TI duration. Sanotra, *et al.* (2001) kept broilers under continuous illumination, which has been demonstrated to increase underlying fear levels and alter TI duration in laying hens (Campo, *et al.,* 2007), and broiler chickens (Onbaşılar, *et al.*, 2008; Zulkifli, et al., 1998), and may have resulted in the differences observed bettween the studies. Vestergaard & Sanotra, (1999) only observed an association bettween TI duration, lameness and TD scores at 38 days of age. As gait scores have been shown to increase with age, the authhours proposed the link bettween fear, gait score and TD observed in their study may have been an indirect effect of a reduction in dust bathing due to lameness at this later stage in the study. Gait score was assessed at 31 days old in the current study, so it is possible an association bettween levels of fear and gait score may have been observed at later ages.

However, the lack of association between gait score or the incidence of TD and TI duration is also consistent with findings by Skinner-Noble & Teeter, (2009) where no differences in measures of well-being, including TI duration, were

associated with broilers assigned gait scores of two or three evaluated with the Bristol gait score. However, Skinner-Nobel & Teeter (2009) removed "birds with obvious leg deformities" from their investigation, though did not provide any detail on their exclusion criteria. Therefore, it is possible that while the birds were assigned scores of 2 or 3 on farm, they may not be a fully representative sample of these scores in practice. This also suggests that while the scoring system of Kestin *et al* (1999) has been found to have good reliability (Garner *et al.,* 2010), the subjective nature of this measure may still lead to discrepancies.

A possible correlation was also hypothesised between tibia breaking strength and gait score as both covary with age and weight (Yalçin, et al., 2001; Estefania, et al., 2019; Shim*, et al.,* 2012). However, no association between gait score and tibia breaking strength was observed. This is consistent with findings by Talaty, *et al.,* (2010) where no association was found with bone mineralisation and gait score. Similarly, Bizeray, *et al.,* (2002) found gait scores were improved in broilers fed sequential meals, though there was no improvement in bone strength associated with this, only a reduction in body weight. Yalcin, *et al.,* (1998) also found no relationship with bone strength and walking ability.

The external validity of gait scoring as a measure of welfare has been well demonstrated for birds obtaining gait scores of ≥3 , which show elevated self-selection of analgesic compared to sound birds (Danbury, et al., 2000), slower walking speeds (McGeown, *et al.,* 1999) a higher incidence of hock burns (Kestin et al., 1999), and have altered behavioural profiles (Weeks et al., 2000; Mench, 2004). However, <1% of broilers in the current study obtained a gait score of ≥3 and differences between lower gait scores are primarily thought to relate to adaptive gait mechanisms based on differences in bird conformation (Paxton, *et al.,* 2013;Skinner-Noble & Teeter,2009), which would be consistent with the finding in the current study where only ADG was correlated with gait score and no other health or welfare indicators.

This suggests that while gait scoring may be a valid and reliable welfare measure overall, it is not necessarily reliably linked with underlying leg pathology or skeletal measures, and that while birds with lower scores may differ in behavioural expression it may not reflect physiological stress or altered affective states.

4.4.c) Associations with Feather Score

No association was found between feather score at 24 days old and TI duration at 29 days old. Previous studies have found associations between poor plumage condition and higher levels of fear (Adams, *et al.,* 1978; Lampang & Craig, 1990; Mahboub, *et al.,* 2004). However, Campo *et al.* (2001) found lower levels of fear in birds with poorer plumage. These inconsistences indicate that feather condition alone may not be a reliable indicator of welfare. This is highlighted by studies where plumage condition was not associated with environmental choice, (Nicol, et al., 2009) and conversely, where hens activley choose to acess additional foraging oppotunites despite higher levels of feather loss and integument damage in these environments; suggesting they were willing to accept this trade off (Nicol, et al., 2011).

However, the studies above have assessed feather cover in laying hens at 40 (Adams, et al.,1978), 72 (Campo, et al., 2001), 57-60 (Lampang & Craig, 1990) and 20-48 (Mahboub, et al., 2004), 20-56 (Nicol *et al.* 2009) and 20-43 (Nicol et al., 20011), weeks of age. Thus, these findings likely reflect the direct impacts of environmental stressors or feather pecking rather than feather growth and development, which was assessed in the current study on broilers chickens at 24 days old.

Additionally, the RSPCA feather score protocol is a single value based on a whole-body assessment of feather cover. Whole body scores have been found to be appropriate for assessing general plumage status, though multivariate analysis which considers specific body parts has been proposed as a more

sensitive analysis for identifying risk factors for plumage damage (Campe, *et al*., 2018).

As discussed in chapter two, feathering rate, feather coverage and plumage quality indicators, such as UV reflectance, may have applications for welfare assessment. However, for feather-based indicators to be a useful welfare assessment technique in commercial settings, a greater understanding   is required regarding the interactions bettween different factors that influence feathering; such as ambient temperature, lighting parameters and nutrition (Leeson & Walsh, 2004a; Leeson & Walsh, 2004b).

Feather development is closely linked to body weight, and is faster in female than male broiler chickens (Wecke, et al., 2017). During an on farm assessment it may not be possible to sex birds while feather scoring them, which means this would be a significant confounding variable for practical applications of feather based measures that should be consisded in future investigations.

4.4.d) Conclusion

In conclusion, the associations in the current study support the idea that gait score is positivley associated with growth rates but not always associated with other bone parameters, TD or underlying fear. Early growth rate was only very weakly associated with tibia strength, though later growth stages may impact this to a greater extent. Fear was also not associated with early growth rates or with feather scoring. Overall this suggests the three welfare measures selected in this study do not covary in a consistent way and that feather score in particular may not be a robust welfare indicator. Further research is needed to determine the validity of fearther scores as a potential welfare indicator in broiler chickens.

VII. GENERAL DISCUSSION AND CONCLUSIONS

The current study investigated the impacts of a white LED lighting system supplemented with UVA or a combination of UVA and UVB wavelengths. A range of performance, health and welfare indicators were measured to establish the impact of UV on these aspects of commercial broiler production. The associations between selected indicators were then examined to investigate the relationships between some of the measures used in the current study.

Broiler chickens treated with only UVA wavelengths had slower initial growth but this had no impact on the end weights obtained. There was no impact on mortality and no improvements in skeletal health indicators. Regardless of this, walking ability assessed using the bristol gait score was improved compared to control broilers. UVA treated broilers also had lower gait scores compared to broilers of similar weights in the control group and exposure to UVA was associated with reduced tonic immobility duration, reduced liklihood of obtaining maximum ti times and fewer TI induction attempts compared to the control group.

Male broiler chickens in the UVA + UVB treated group grew faster than control broilers and obtained similar final weights. Walking ability was improved in the UVA + UVB treated group, with heavier birds obtaining lower gait scores than control broilers of similar weights.

Bone parameters and Tibial Dyschondroplasia (TD) were not associated with higher gait scores, and there was no difference in the severity of TD in the different treatments. No ocular abnormalities were discovered as a result of the lighting treatments used in the current study.

The results of the current study are in agreement with other investigations of LED systems with different wavelength compositions. Monochromatic blue LEDs or White LED systems with increased power outputs of shorter wavelengths, have been shown to reduce fearfulness and stress while

improving performance (Archer, 2015; Archer, 2016; Mohamed, et al., 2017; Archer, 2018). However, other studies have also found no differences in welfare and stress parameters between different white LED colour temperatures (Olanrewaju, *et al.,* 2015; Riber, 2015). The UVA LED used in the current study also increased the amounts of violet and blue wavelengths compared to the control group. More recently , studies utlising UVA LEDs have found reductions in fearfullness in broiler chickens (House*, et al.,* 2020) and laying hens (Sobotik, *et al.,* 2020) in addition to reduced baseline corticosterone, lower bilateral asymmetry and lower heterophil lymphocyte ratios, indicating a role of UVA in altering fear and stress responses.

Both of these studies used UVA LEDs with a slightly lower peak output of 380nm. Additionally the white LEDS used by House*, et al.* (2020) had a larger power out put in the blue part of the spectrum compared to the white LED light of the current study. As differences in fear and stress were still reported, this could potentially, indicate that the beneficial impacts on fear found in the UVA only treatment do relate to impacts of UVA wavelegnths, rather than simply blue wavlengths.

Therefore, further investigation to determine the mechanisms by which blue and UVA light act to reduce underlying fear and stress would be of great importance to the poultry industry. Further comparisons between UVA emitting LEDs and white LEDs with additional blue wavelengths (but no UVA) will allow for greater distinction between the effects of different wavelengths on welfare, health and performance indicators.

A potential mechanim worth further investigation is photostimulation of non-visual photoreceptors in the retina, pineal gland and hypothalamus; which regulate a range of essential neuroendocrine pathways. In particular, blue light has already been shown to alter circadian melatonin amplitude (Jin, *et al.,* 2011), and attenuate the stress response in broilers (Saito, *et al.,* 2005), subsequently improving cellular and humoral immune responses (Xie, *et al.,* 2008).

As UVA has also shown to be a powerful entraining stimulus able to inhibit melotonin synthesis (Zawilska, *et al.,* 2007), so the implications of this and potential different roles of UVA and blue wavelengths would be an important area of further study.

One of the limitations of the current study was that behavioural welfare indicators and space use (to determine the use of UV in treatment B) were not measured. A range of bird species have demonstrated a preference for light environments with UVA (Ross, *et al.,* 2013) and indicated a potential impact on exploratory behaviour (Maddocks, *et al.,* 2001). Further investigation of this in chickens with updated lighting technology would help determine if UVA wavlengths have other psychological value such as impacting environmental choice or response to novelty.

One of the benefits of LED systems is that they allow for relatively precise control over the spectral composition of the light environment. This makes them ideal for use in further studies investigating the benefits of different wavelengths, in order to establish lighting regimes maximising the performance health and welfare of broiler chickens.

The effects of combined UVA + UVB may also a promising area for further study. While there were no improvements in bone mineral density assessed using Dual Energy X-ray Absorbimetry (DEXA). Other measures of bone quality, skeletal architecture, or body conformation may reveal other mechanisms for the improvements in walking ability at heavier body weights observed in treatment B in the current study.

Both UVA only and a combination of UVA + UVB improved the walking ability of broiler chickens when assessed using the Bristol gait score (Kestin, et al., 1992). Gait Scores were worse in heavier birds, though there was also an interaction effect between treatment and weight, with heavier birds in the UV treated groups having lower (better) gait scores than control birds of similar weights (section 2.3.c). This effect is highlighted by an overall correlation observed between gait score and average daily gains during early growth in

this study (section 4.3), where a weak positive correlation between higher (worse) gait scores and higher average daily gains was observed. While lower gait scores may not represent a difference in stress or wellbeing (Skinner-Noble & Teeter, 2009), improved mobility still allows birds to engage in positive behaviours that may otherwise be too energetically expensive (Vestergaard & Sanotra, 1999; Weeks, et al., 2000). The improvements in mobility found in the current study are worth further investigation as it indicates lighting manipulations such as UV supplementation may be able to alter the trajectory of decreasing activity levels and higher gait scores that are commonly reported in the literature (Su, *et al.*, 1999; Kestin, *et al.,* 1991; Kestin, *et al.,* 2001; Sanotra, *et al.,* 2001; Kristensen, *et al.,* 2006; Paxton, et al., 2013; Tickle, et al., 2018).

This is also highlighted by the faster growth of male broilers in the UVA + UVB group. Despite reaching finishing weights early than control birds, this was not accompanied by declining mobility to the same extent as control broilers, indicating scope to improve mobility without an inevitable reduction in performance.

Conversely, broiler chickens in the UVA only treated group had reduced growth, despite obtaining similar end weights. While this was not associated with any improvements on skeletal parameters, further studies would be warranted to determine if this was related to changes in behaviour or activity.

It was hypothesised that UVB provision may improve walking ability through providing opportunities for endogenous Vitamin D synthesis, leading to improvements in skeletal health, such as improved bone mineral density and bone strength. However, Texture analysis, Dual Energy X-ray Absorptiometry (DEXA) analysis and bone measurements revealed no biologically relevant improvements in skeletal parameters. This is in agreement with the results of Talaty, et al. (2010), where bone mineral density had little impact on the walking ability of broiler chickens.

Previous studies investigating the potential of UVB to improve skeletal measures used imbalanced or deficient diets (Edwards, 2003; Fleming, 2008). However, broilers in the current study were fed a commercially representative diet, which may have limited the potential to improve skeletal health. Another limiting factor could be the genotype of the broilers themselves, as TD is hereditary and may be associated with impaired vitamin D metabolism or expression of vitamin D receptors (Mitchell, *et al.*, 1997). Further investigation into the vitamin D status of broilers treated with UVB, in addition to other measures of health and immune function would help determine the value of UVB for improving the performance health and welfare of broiler chickens.

The current study incidates this may not be a commercially viable way reduce lameness and support skeletal development, but due to the shorter half-life of dietary vit D supplementation, other benefits to health parameters not measured in the current study such as immune function may be worth further investigation with an altered experimental set up where more birds are able to acess UVB exposure. The main contributions of the current study disscussed above are summarised in figure VIII.I

A) Investigating the impacts of UV supplementation

Compared to white LED control:

- +UVA reduces fearfulness
- +UVA improved mobility
- +UVA & UVB improves mobility
- +UV no impact on final weights
- + UVA reduces early growth rates
- +UVA & UVB increases early growth in males
- +UV no impact on skeletal measures/ TD
- +UV no impact on ocular health

A)

Animal Managment and Husbandry

Data Collection

Evidence based husbandry changes

Welfare Assesment Tools and Techniques

B)
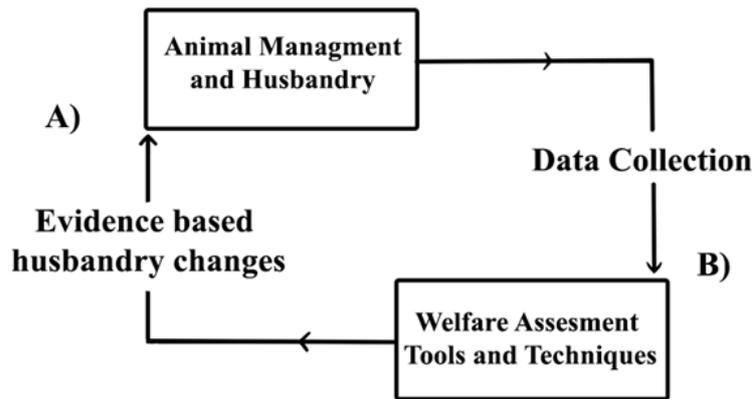
B) Associations between indicators which add to existing literature on welfare assessment

- Association found between gait score and weight and gait score and early growth rate.
- No association between gait score and measures of fear, bone parameters or TD.
- Feather score was affected only by sex and weight and was not associated with levels of fear. Feather scoring requires further validation before use as a welfare indicator.

Figure VII.i- summary of main findings

The main findings are contextulised according to how they contribute to two complementary aims for improving the welfare of production animals (detailed in section V.II) **A)** Evidence that may contribture to changes in animal husbandry is highlighted for UV supplementation (+UV) including treatment A (+UVA 18 hours) and treatment B (+ UVA & UVB 8 hours), **B)** Information that contributes to existing liturature on welfare assesment is highlighted, including associations bettween other performance and health measures such as tibial dyschondroplasia (TD)

The challenges of implementing UV supplementation on a commercial scale include the relatively high cost of UV emitting light sources compared to conventional light sources. Cost-benefit analysis would be required to establish the long-term value of UV light supplementation and encourage uptake of UV light sources in commercial practice if further benefits to bird performance, health and welfare are established. Fluorescent tubes also only emit UVB for 12 months before they require replacement, so a cost-benefit analysis would need to factor in the time, labour and cost of replacing bulbs.

There are also a number of different ways to provide UV supplementation which would require testing to determine their effectiveness in commercial practice. Light sources that provide UVB typically do not emit them over large distances, with T5 fluorescent tubes offering the widest distribution of UV (UV index of 0.5 measured 1.2m from the bulb) (Baines, *et al.,* 2016). The implications of this is that, for UVB provision, bulbs would need to be installed relatively close to ground level, and care must be taken to ensure their instalment still allows for easy cleaning between flocks of chickens and that the units can withstand the heat, humidity and dust produced within poultry sheds.

An alternative method would be using transparent materials that transmit UV for poultry shed windows or roofing, such as Ethylene tetrafluoroethylene (ETFE). However, this would make UV exposure more unpredictable (with seasonal variation) and if windows are shut to reduce light intensity and control feather pecking there would consequently be no UV provision, making the costs and benefits of this method harder to predict.

The current study found promising results at a commercially representative stocking density of 33kg/m2. However, stocking densities can exceed this, and results may be more variable with the much larger flock sizes in commercial poultry houses.

The current study measured a number of performance, health and welfare indicators to establish the impacts of UV supplementation on these key areas of poultry production. While a large number of indicators were measured, feed

conversion data, vitamin D status, activity levels and behavioural data would be of further importance for future studies.

In conclusion, UV wavelength supplementation represents a promising husbandry refinement, offering benefits to broiler chicken welfare without reducing performance.

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IX. APPENDIX

IX.I) Flow charts for statistical analysis

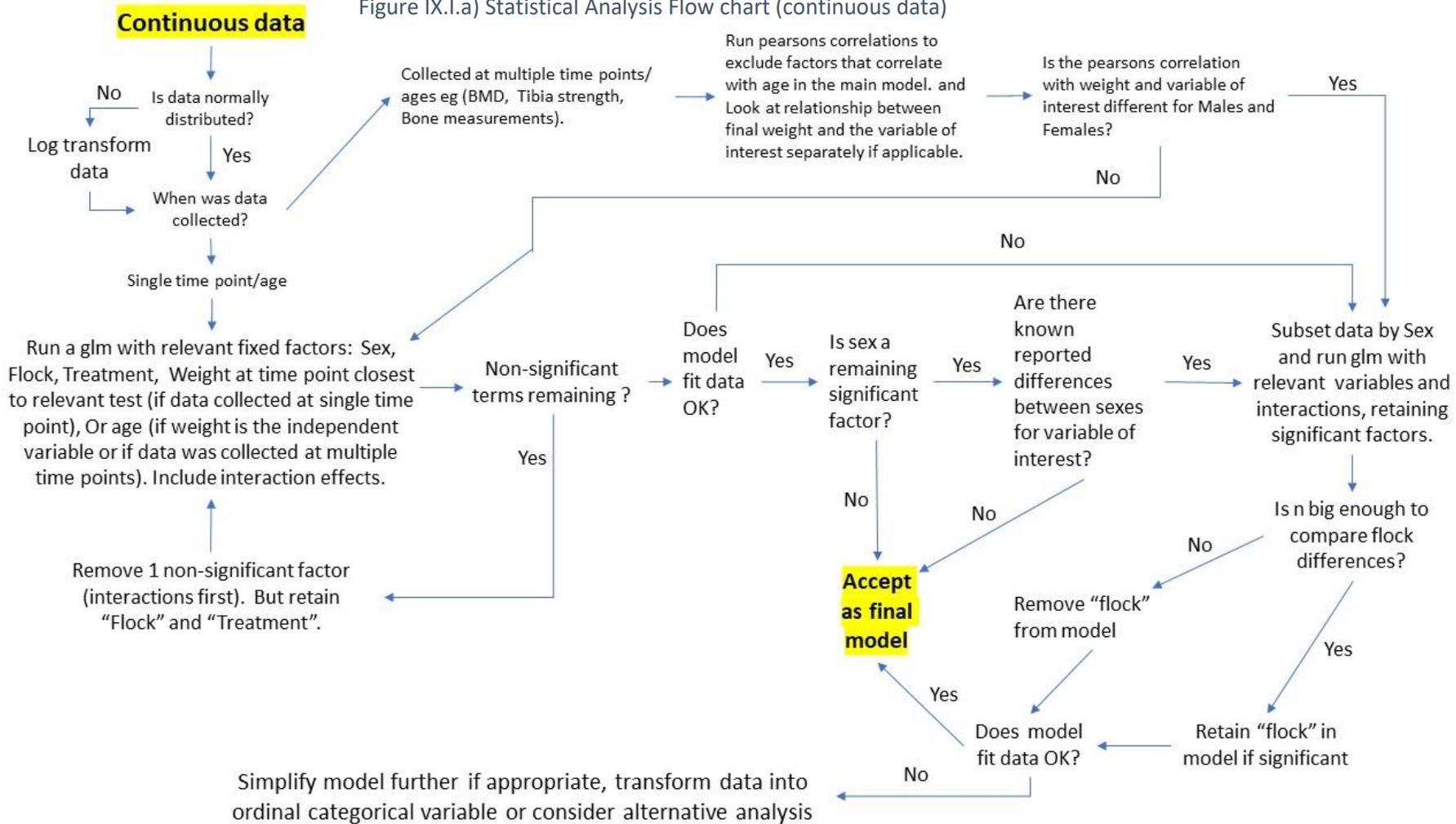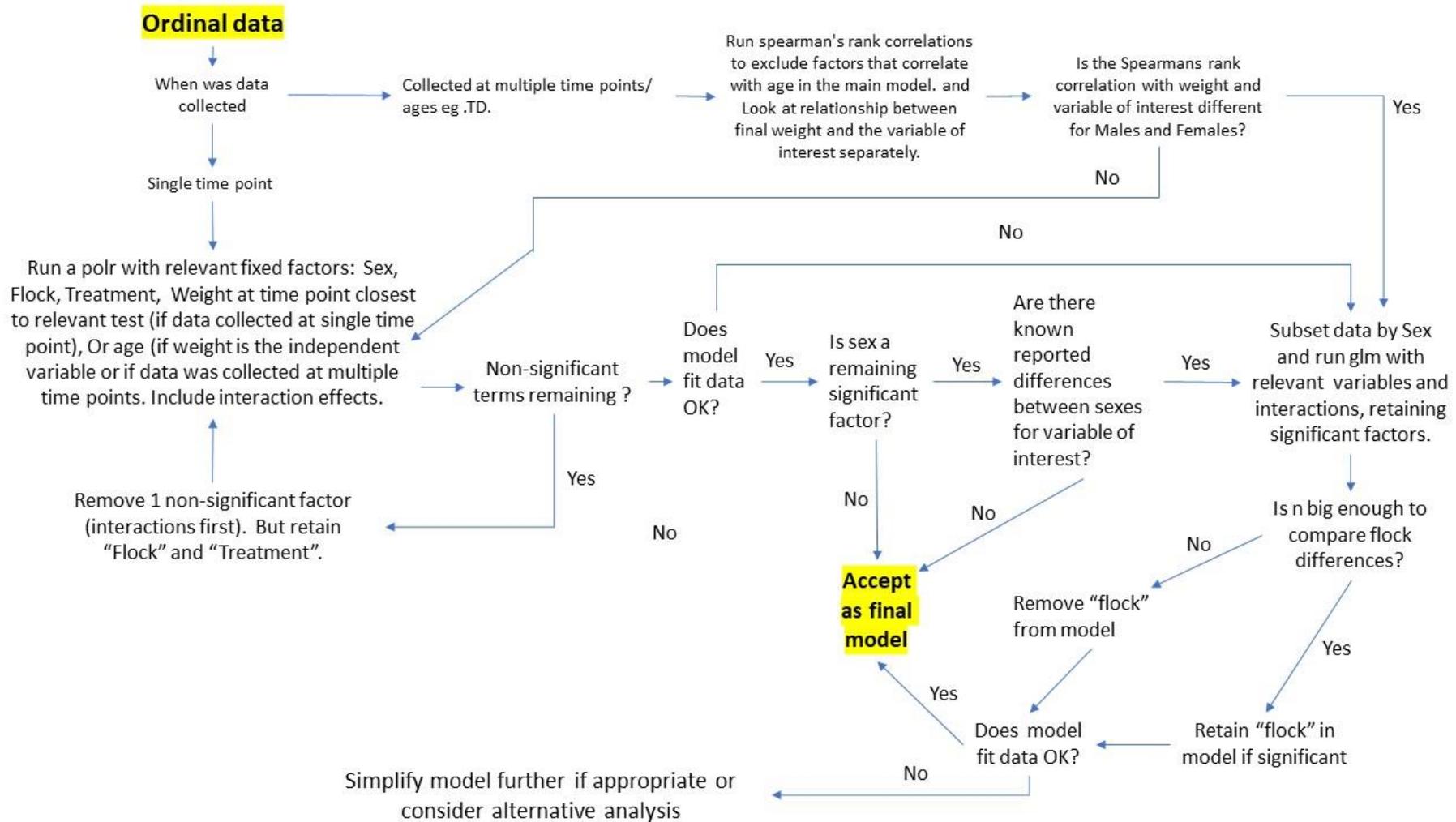Figure IX.I.a) Statistical Analysis Flow chart (continuous data)

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Figure IX.I.b) Statistical analysis flow chart (categorical data)

IX.II) Haematoxylin and Eosin Staining Procedure

Slides were first washed in tap water to remove residual OCT compound and stained in 1% Harris Haematoxylin (VWR chemicals) for 5 minutes. This was followed by washing with running tap water and de-staining in 1% acid alchohol for 10 seconds. Slides were then washed in Scott's tap water (made with 1 litre of distilled water mixed with 20g Sodium bicarbonate and 3.3g Magnesium Sulphate) and stained in 1% eosin (RAL diagnostics) for 20 minutes followed by washing in tap water.

IX. III) Alcian Blue Staining Procedure

### Reagent preparation for 1% alcian blue solution pH 2.5

3% acetic solution was prepared by adding 15 mL glacial acetic acid (Fisher scientific, A/0400/PB17) to 485 mL distilled water. Subsequently 5 g of Alcian blue 8GX powder (Sigma, A 3157) was added. The solution was mixed and filtered using Whatman filters no.1 (Cat no. 1001 150). The reagent pH was adjusted to 2.5 using 1M sodium hydroxide (Sigma, S8263).

Reagent preparation for 0.1% Nuclear Fast Red, 5% aluminum sulfate solution

25 g aluminum sulfate (Aldrich chemistry, 202614) was dissolved in 500 mL distilled water on an electronic stirrer. Subsequently 0.5 g Nuclear Fast Red (Sigma 60700) was added, and left on hot plate stirrer until the solution had boiled and all ingredients completely dissolved. The solution was left to cool at room temperature and then filtered using Whatman no.1 filters.

Alcian Blue Staining Procedure

Slides were washed in tap water to remove OCT residue before being stained in 1% Alcian blue (Ph 2.5) for 5 minutes, washed in tap water and then stained in nuclear fast red for 5 minutes.
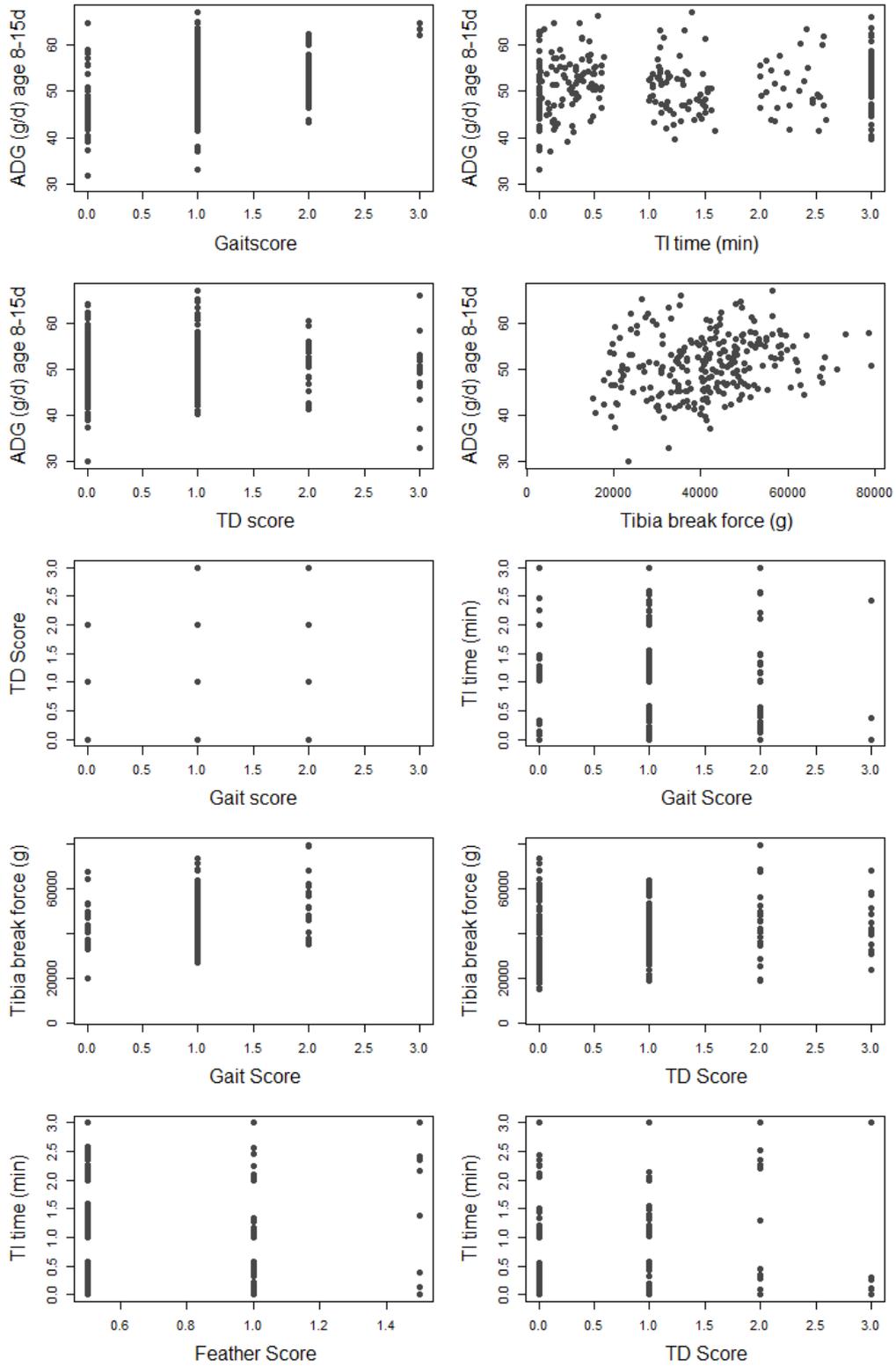
IX. IV) Associations among selected variables (chapter 4)

X, Y scatter plots of selected performance, health and welfare measures.

TD = Tibial Dyschondroplasia, TI = Tonic Immobility, ADG = Average daily gains

Name of Organization

Animal and Plant Health Agency, Weybridge, Surrey

Details of Placement

Please describe your main activities during the placement

During my placement, I worked at the Virology department of the APHA. My project was to assess the effectiveness of nasal wipes for detecting viral shedding from pigs infected with influenza virus compared to the gold standard method of using nasal swabs. Nasal swabs are a home office regulated procedure which require animals to be restrained. This is stressful for the animals and requires several trained technicians to take samples. Nasal wipes can be performed quickly without restraining the animal and may be a more favourable experience for both animals and staff.

My activities included; (a) Researching what materials to use when sampling pigs (b) performing a materials test by spiking materials with influenza virus, extracting viral RNA and quantifying the results using real time RT-PCR. (c) coordinating and carrying out sampling of infected pigs using the selected materials, (d) processing samples while working in microbiological safety cabinets, where virus was re-suspended in media and RNA was extracted and quantified with RT-PCR. (e) analysing and presenting the results at the virology department lab meeting and to the animal handling staff.

Placement Achievements

Please detail all outcomes from the placement, including any publications, presentations given, and reports written etc.

My achievements and outcomes include:

- Showing that nasal wipes represent a promising animal welfare refinement for the sampling of influenza virus in pigs.
- Presenting these findings at the APHA Virology departments lab meeting, Preparing a lab report for the APHA of the methods and findings.
- Presenting a subsequent talk on the PIP outcomes and my own research at the APHA's Species Group Care & Use Committee Meeting (18th May 2016).
- Presented a poster of the outcomes of the PIP placement at 7th International Conference on the Assessment of Animal Welfare at Farm and Group Level in the Netherlands 5th-8th September 2017.

Skill development

Has this Placement helped you developed any new skills or enhanced your previous skill set?

I learnt new technical skills including; working in microbiological safety cabinets with live virus samples, performing manual spin column or robot viral RNA extractions and Real Time RT-PCR techniques. In addition to developing skills with practical virology work I received home office training for pigs and ferrets. I was also able to learn more about the applications of positive reinforcement training for animals used in research, both practically with the pigs and on a trip to the Envigo primate facilities in Huntingdon arranged through the APHA. I gained further experience with project planning and experimental design, problem solving, troubleshooting and protocol optimisation within a short 3-month time span.

Future Work

Has this Placement influenced your future career aspirations? If so, in what way?

It was valuable to gain experience working with a government research facility. I have built up a network of contacts within the APHA which has helped me consider different career options after completion of my PhD.

Give brief details of any other work undertaken during your Doctoral Training Partnership.

At the start of my Doctoral training partnership I undertook three projects;

- Investigating different trapping methods for estimating harvest mice (*Micromys minutus)* populations in reed bed habitats.
- The application of LEDs in Vertical farming
- The ecological consequences of immune variation in wild mouse populations (M*us musculus*)

These projects allowed me to gain further technical skills and analyse a range of different kinds of data including; Conducting ecological surveys, small mammal trapping, small mammal dissection, performing ELISAs for detecting serum IgG and IgE antibodies, parasite identification, Flow cytometry and fluorescence activated cell sorting (FACS analysis), 3D modelling of plant canopies and measuring photosynthesis using infra-red gas analysis.

I also conducted an experiment on a commercial broiler chicken farm investigating the applications of thermal imaging and gait analysis for the objective assessment of lameness in comparison to the Bristol gait scoring system. This work is being written up for publication separately.