The impact of dark exposure on the feeding behaviour, production, and gastrointestinal tract segment and content weights of broiler chickens

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Overall Abstract

Broiler feeding behaviour was observed at two ages to quantify how varying durations of darkness alter behaviour and impact productivity and alterations in the gastrointestinal tract (GIT) over 24 h. The impact of dark exposure on productivity, GIT segment and content weights and feeding behaviour of Ross 308 broilers (7-31d) was studied. Four lighting programs were used (23L:1D (1D), 20L:4D (4D), 17L:7D (7D), and 14L:10D (10D). The birds (n=4000) were housed in 8 rooms with 8 pens per room (2 replications per lighting treatment and 4 replications per gender per room). The GIT data were collected on d 27-28 (6 males per lighting program, euthanized at 2 h intervals for 24 h). Production data were analyzed using SAS Proc Mixed as a 4 (dark) x 2 (gender) factorial arrangement and GIT segment data as a 4 (dark) x 12 (time) factorial arrangement, with lighting program nested within room. Dark data were analyzed using regression analyses and analysis of variance. Differences were significant when $P \leq 0.05$. At 31 d, regression analyses showed no effect on body weight, however numerically birds raised on 4D and 7D were heaviest. The highest feed consumption was observed under 4D. Birds on 10D were the most feed efficient (linear response). A quadratic effect on mortality was found, with the highest mortalities under 4D and 7D. Birds on 10D had the heaviest empty crops (% of body weight (BW). Crop content (% BW) changed quadratically, with peaks prior to dark under 4D, 7D, and 10D, suggesting anticipation of darkness. The empty gizzard weight (% BW) increased linearly as dark increased. Behaviour was examined as a 4 (dark) x 2 (age (2, 4 wk) x 2 (gender) factorial arrangement with lighting program nested within room. Five males or females per room were marked and focally observed. Statistical analyses were performed similar to the production data. As dark increased, feeding bout frequency increased and feeding bout interval decreased linearly. Total time spent at the feeder decreased linearly as dark increased. As birds aged, feeding frequency decreased and feed bout length and interval increased. Males visited the feeder more frequently and had shorter bout intervals. Birds anticipated dark periods >4 h and increased their feeding activity prior to dark. Broilers adapt their feeding behaviour in response to dark exposure, which alters GIT segment and content weights, and likely feed passage rates, in turn affecting feed efficiency and digestibility.

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Table of Contents

List of Tables

List of Figures

List of Abbreviations

D	**dark**
d	**day**
EEG	**encephalogram**
FCR	**feed conversion ratio**
G:F	**gain-to-feed ratio**
$G:F^m$	**gain-to-feed ratio corrected for mortality**
GIT	**gastrointestinal tract**
H:L	**heterophil to lymphocyte ratio**
L	**light**
TD	**tibial dyschondroplasia**
TI	**tonic immobility**
VVD	**varus-valgus deformation**

1.0 Chapter 1. Literature review: The impact of dark exposure on nutritional behaviours, productivity traits, and changes in the gastrointestinal tract of broiler chickens

1.1 Introduction

Producers are beginning to understand that from a production, health and welfare standpoint, darkness is an important management tool and the implementation of lighting programs that incorporate periods of darkness are becoming more common in the poultry industry. Due to the importance of lighting programs as a management tool in the broiler industry, Canada has recently updated its requirement for photoperiod duration (NFACC, 2016). The National Farm Animal Care Council's current Codes of Practice for the Care and Handling of Hatching Eggs, Breeders, Chickens and Turkeys requires producers to gradually increase the amount of darkness in every 24 h period from 0 h to a minimum of 4 h by day 5 and maintain this minimum level of darkness until at least 7 days (d) prior to catching (NFACC, 2016). Legislation from the European Union requires producers to provide, at minimum, a total of 6 h of darkness in every 24 h period, with at least 4 h of continuous darkness, after the first 7 d and until 3 d before slaughter for broilers (European Commission, 2007). The period that lights are turned on is referred to as the photoperiod, whereas the period of time that lights are turned off is known as the scotoperiod.

One of the most important economic aspects of commercially raising poultry is to achieve a desired body weight, efficiently, in a relatively short time period. Producers monitor feed intake, feed efficiency and body weight to ensure birds are performing at their genetic potential. Production parameters can also be useful in monitoring the welfare of a flock, however this should never be the only measurement of bird welfare.

Recently animal welfare has become one of the largest consumer concerns facing the livestock and poultry industries in many areas of the world. Key components of animal welfare are basic health and functioning, affective states, the expression of natural behaviour and the ability of an animal to adapt to its environment (Duncan, 2002; Fraser, 2008). One set of management standards that has become almost synonymous with animal welfare are the "Five Freedoms", which were originally developed in the Brambell Report (1965) in response to concerns about the welfare of intensively farmed animals in the United Kingdom. Since that time the freedoms have been revised by the Farm Animal Welfare Council (FAWC, 2009) to be more comprehensive and to ensure welfare is being maximized. The revised Five Freedoms are:

1. Freedom from hunger and thirst: by ready access to fresh water and a diet to maintain full health and vigor.
2. Freedom from discomfort: by providing an appropriate environment including shelter and a comfortable resting area.
3. Freedom from pain, injury or disease: by prevention or rapid diagnosis and treatment.
4. Freedom to express normal behaviour: by providing sufficient space, proper facilities and company of the animal's own kind.
5. Freedom from fear and distress: by ensuring conditions and treatment which avoid mental suffering.

With these Freedoms in mind, to effectively assess welfare, various parameters should be monitored including those relating to production, health and affective state of the bird, using behaviour as a monitoring tool. Welfare is important not only for the birds themselves, but it is also important to producers because reduced welfare may compromise bird productivity or the producer's economic return. Other stakeholders who are invested in a high quality of welfare are consumers, and the consumer demand for a higher standard of welfare influences primary producers, processors and retailers.

Consumers have become concerned with the state of welfare that broilers experience throughout their lives, including during rearing, catching, transportation and slaughter. While welfare can be difficult to measure, one common component of assessing animal welfare is the observation of behaviour (Duncan, 1998; Duncan 2005). For example, quantifying how animals allocate their time between different activities can suggest which behaviours are important to the animal. Certain behaviours, including those necessary for survival, are driven by strong internal and external motivators and a reduction or elimination of these behaviours results in reduced welfare (Duncan, 1998; Prescott et al., 2003). Nutritive behaviours, including feeding and drinking, are necessary for survival and are highly motivated in poultry (Duncan, 1998; Bokkers et al., 2004). One method used to assess the motivation an animal has to perform a specific behaviour is to use a preference test (Hughes and Duncan, 1988). Preference tests, using feed restricted broiler breeders, have shown feeding to be a highly motivated behaviour because birds were willing to work or pay a "cost" to obtain feed (Dixon et al., 2014). A reduction in highly motivated behaviours may however be independent of welfare, which makes interpretation on its own difficult. For example Dawkins (1990) explained that a reduction in nutritive behaviours could be due to the stage of an animal's life, so proper understanding and interpretation of behaviour is required. In addition to assessment of welfare, behavioural research also provides useful insight into other possible concerns, including animal health, production and condemnations.

The impact of providing darkness for chickens has been well studied (Classen, 2004; Brickett et al., 2007; Onbasilar et al., 2008; Olanrewaju et al., 2012; Schwean-Lardner et al., 2012b, 2013; Yang et al., 2015), however few research programs have focused on behaviour (Sanotra et al., 2002; Lewis et al., 2009a; Bayram and Ozkan, 2010; Schwean-Lardner et al., 2012a, 2014). Instead, often the focus has been on bird productivity and these results have been variable. Results of preference tests where broilers were permitted to choose the duration of darkness they were exposed to indicated a preference for 4 hours (h) in a 24 h period (Savory and Duncan, 1982). Modern broilers may have a different preference and it must also be noted that a bird's preference does not always correspond to what is optimal for them in terms of productivity, health or welfare. Exposing birds to different durations of darkness affects their productivity, health, welfare and behaviour, and therefore the effect of different durations of darkness needs to be evaluated to ensure birds are performing at their potential while optimizing their well-being.

Examination of chicken behaviour under different lighting programs has only studied behaviour during a portion of the day period (Malleau et al., 2007; Bayram and Ozkan, 2010) and when behaviour was examined over 24 h, scan sampling was often the technique used (Schwean-Lardner et al., 2014). Previous studies have also compared one lighting program to another (Sanotra et al., 2002; Lewis et al., 2008), however few examined the relationship between photoperiod length and the variable being studied (Schwean-Lardner et al., 2012a,b, 2013, 2014). The research presented in this thesis differs from previous works in that behaviour was monitored continuously over a 24 h period using focal sampling. The changes in behaviour were then linked to changes in production and gastrointestinal tract segment and content weights.

1.2 Impact of dark exposure on productivity

Constant (24 h light) or near constant lighting programs (23 h light and 1 h dark (23L:1D) have been used during the rearing phase of broilers (Chicken Farmers of Canada, 2009). Reasons for this included easier catching at the time of shipping due to lethargic birds and for 23L:1D acclimating birds to darkness in the event of a power outage (Savory and Duncan, 1982). Another reason producers implemented continuous or near-continuous lighting programs was to provide birds constant access to feed to facilitate maximum feed intake and increase body weight (Savory, 1976; Gordon, 1997; Lewis and Morris, 2006). Many studies have reported results that confirm this idea, however these studies often compare programs with either relatively short (23L:1D) or long (12L:12D) scotoperiods, without examining the effect of moderate dark periods (Brickett et al., 2007; Abbas et al., 2008). Studies by Schwean-Lardner et al. (2012a,b; 2013; 2014) examined four lighting programs (23L:1D, 20L:4D, 17L:7D and 14L:10D) and found that providing a moderate dark period had a positive impact on a variety of production, behaviour and welfare parameters. The following sections will examine the impact of increasing levels of darkness on different production variables.

1.2.1. Growth Rate

When broilers are raised with a dark period included in their lighting program, a shift in their growth curve occurs that results in less body weight gain during the early stages of life. However, compensatory growth often results in equal or greater final market body weights (Classen, 2004). This was shown in a study by Bayram and Ozkan (2010) who found that broilers raised on continuous light were heavier than those raised on 16L:8D at 3 weeks (wk), but by 6 wk there was no significant difference in body weight among lighting treatments. A reduced early body weight is beneficial to the bird because it allows for development of the skeletal (Robinson et al., 1992) and cardiac systems (Classen and Riddell, 1990) at a slower rate before the heavier body weight is deposited. Classen (2004) looked at the effect of lighting program on performance measures and found that body weight at 15 d was highest in birds raised on 16L:8D and 20L:4D compared to 12L:12D. By 35 d the heaviest birds were those raised on 20L:4D, followed by 16L:8D and the lightest birds were those raised on 12L:12D. These results indicate that while a certain amount of darkness is beneficial to birds in terms of body weight gain, a period of darkness that is too long may not be optimal. However, this likely depends on the market age of the birds and may not be the case for birds reared to heavier weights, for example those slaughtered at 56 days of age. Schwean-Lardner et al. (2012b) measured body weights of broilers and found the heaviest birds were those raised under 20L:4D at both 32 and 39 d compared to birds reared on 23L:1D, 17L:7D or 14L:10D. At 49 d, body weights under both 17L:7D and 20L:4D were significantly heavier than under 14L:10D and 23L:1D. From the above data, one could infer that periods of darkness greater than 7 h may result in negative effects on body weight.

Lewis et al. (2008) examined the effect of lighting program on male broiler performance, using continuous lighting for the first day of age before starting lighting programs on d 2. The lighting programs used were 8L:16D, 16L:8D or an initial 8 h light then transferred to 16 h at d 10, 15 or 20. The authors did not find a difference in bird body weight between the treatments at any of the periods measured (21 d, 22 to 35 d or 35 d), which is likely due to compensatory gain occurring by 21 d when body weights were first recorded. These data were then combined with a prior study examining the impact of the same lighting programs on productivity with female broilers (Lewis and Gous, 2007) and the pooled data showed that for the first 21 d body weight did not differ between treatments. However, from 22 d to 35 d (males) or 42 d (females) the birds raised on 8L:16D were heavier compared to 16L:8D, but did not differ from birds transferred from 8L to 16L at 20 (males) or 21 (females) d of age. The same effect was observed for feed intake and efficiency during this period, which would contribute to the observed increase in body weight. A possible explanation for birds raised under 8L:16D having a higher final body weight than birds raised under 16L:8D was that birds raised on the longer dark period learned to feed during the dark period. Previous work has shown negligible feeding occurring during dark periods unless those dark periods are longer than 12 h (Lewis et al., 2009a).

1.2.2. Feed intake

Modern broilers have been selected for increased feed intake and are highly motivated to feed (Bokkers and Koene, 2004). Feed intake is an important measurement to assess, not only from a productivity standpoint but also in terms of behaviour and welfare. Birds rely heavily on vision to identify and locate feed which is why, traditionally, broilers have been raised on continuous or near-continuous lighting, allowing them increased visual access to feed. Research has shown that although exposure to longer daylengths does facilitate higher feed intake, photoperiods, as well as scotoperiods that are too long may result in negative effects. Classen (2004) found that feed intake was highest in birds raised on 20L:4D from 0-15d, 15-35d and overall from 0-35d, as compared to the 16L:8D and 12L:12D treatments. Schwean-Lardner et al. (2012b) used scotoperiods consisting of 1D, 4D, 7D and 10D and results showed feed intake was highest under 20L:4D for birds raised to 39 or 49 d. Birds raised under 23L:1D consumed as much (39 d) or less feed (49 d) than birds raised under 17L:7D.

In a study comparing broilers reared under 8L:16D or 16L:8D, it was found that from 0-21 d birds raised on 16L:8D had higher feed consumption, but from 22-35 d (males) or 22-42 d (females) birds raised on 8L:16D had a higher feed intake (Lewis et al., 2008). The authors suggested this could be because the birds learned to feed during the scotoperiod due to a higher feed requirement as the birds aged. At the end of the trials (35 or 42 d) there was no difference observed in feed intake between birds reared on either lighting program, again suggesting that birds raised on 8L:16D were likely feeding during the scotoperiod to meet their feed requirements. Bayram and Ozkan (2010) also saw no difference in feed consumption between broilers raised on 24L:0D and 16L:8D. Schwean-Lardner et al. (2012a) reported that birds did not visit the feeder during the dark period, except for those raised on 14L:10D who fed a negligible amount. The authors attributed this nocturnal feeding to either birds experiencing hunger or no longer requiring sleep/rest for that night. A possible explanation for why birds do not normally feed during the scotoperiod is that melatonin, which peaks during darkness, suppresses feed intake (Bermudez et al., 1983).

1.2.3. Feed efficiency

Research has shown that the length of the scotoperiod can impact feed efficiency in broilers. Schwean-Lardner et al. (2012b) found that gain-to-feed (G:F) efficiency responded in a quadratic fashion, with maximum efficiency observed under the longest scotoperiod, which was 10D. Classen (2004) reported that feed-to-gain (F:G) and feed-to-gain corrected for mortality

($F:G^m$) responded in a linear fashion with improved efficiency as the length of the dark period increased. Lewis et al. (2008) found no differences in broiler feed efficiency from 0-21 d, but from 22-35 d (males) or 22-42 d (females) broilers raised on 8L:16D were more efficient than those reared under 16L:8D.

Birds raised on longer dark periods may have improved feed efficiency for a number of reasons. One explanation is that these birds are less active during the dark period (Alvino et al., 2009; Blatchford et al., 2009), and may instead use this time to rest/sleep. Broilers exposed to dark periods show an increase in endogenous melatonin levels, which may relate to a decrease in heat production due to minimal night-time activity which results in a lower energy expenditure (Apeldoorn et al., 1999). Another explanation is that the diurnal rhythm of body temperature reduces metabolic rate during the dark (MacLeod et al. 1980; Brickett et al., 2007), which may result in a lower maintenance energy requirement (Classen, 2004). Although the majority of research has concluded that darkness improves feed efficiency there are some exceptions. For example a study by Bayram and Ozkan (2010) found that the feed conversion ratio (FCR) did not differ between birds raised on 24L:0D or 16L:8D from 0-3 or 3-6 wk. The authors did not measure feed wastage and suggest it could partially account for the lack of an effect.

1.3 Impact of dark exposure on bird health

Lack of darkness may have a number of negative implications for bird health, based both on biological and physiological processes. Providing birds with a period of darkness resulted in improved metabolic health (Schwean-Lardner et al., 2013), and immune response (Kirby and Froman, 1991; Gordon, 1994; Rozenboim et al. 1999), decreased the incidence of skeletal disorders (Classen and Riddell, 1989; Classen et al. 1991; Schwean-Lardner et al., 2013), altered ocular development (Lewis and Gous, 2009; Schwean-Lardner et al., 2013; Leis et al., 2016), increased resting behaviour (Malleau et al. 2007; Schwean-Lardner et al., 2014), increased overall activity (Sanotra et al., 2002; Schwean-Lardner et al., 2012b) and reduced overall mortality (Brickett et al., 2007; Schwean-Lardner et al., 2012a). Continuous or near-continuous lighting programs are thought to result in an increase in the incidence of diseases that are partially due to rapid growth including ascites, sudden death syndrome (SDS) and skeletal issues

(Classen and Riddell, 1989; Classen et al. 1991; Lewis et al., 2009a; Renden et al., 1991; Schwean-Lardner et al., 2013). Health can also be effected by the tissue rejuvenation that occurs while birds are sleeping (Malleau et al., 2007). Therefore, providing birds with a period of darkness, which slows early growth, increases exercise and alters physiological health is beneficial. Health problems reduce bird welfare and may result in reduced flock feed efficiency. They can also result in economic losses due to on farm culling and/or an increase in the number of condemnations/downgrading at the processing plant (Brickett et al., 2007; Malleau et al., 2007).

1.3.1. Skeletal health

Exposure to long photoperiods increases the occurrence of leg abnormalities (Classen and Riddell, 1989; Classen and Riddell, 1990; Sanotra et al., 2002; Schwean-Lardner et al., 2013), whereas periods of darkness provide a time for birds to rest, uninterrupted, which may be one mechanism that results in a positive effect on skeletal growth (Brickett et al., 2007; Schwean-Lardner et al. 2014). Although genetic selection has reduced the number of leg defects in broilers overall (Classen, 2004), research still demonstrates that the addition of a scotoperiod into a photoperiod program further reduces skeletal defects as compared to when birds are reared under constant or near-continuous light (Sanotra et al., 2002; Schwean-Lardner et al., 2013). Previous research has pointed to a number of mechanisms by which this may occur (Renden et al., 1996; Classen, 2004; Brickett et al., 2007; Schwean-Lardner et al., 2013).

Rapid growth rate at a young age results in an increased weight load on the immature skeleton of young birds, which may contribute to a higher incidence of leg disorders (Julian, 1998). In a previous section, evidence was given that the use of a dark period changes the growth curve of broilers, with slower growth occurring early in life as the skeletal structure is still developing, resulting in more stable bone development (Sanotra et al., 2002). This is then followed by a period of compensatory growth, which results in market body weights equal or greater to that of birds raised under constant or near constant light (Sanotra et al., 2002; Classen, 2004; Schwean-Lardner et al. 2012b).

Bone strength is effected by bone matrix volume and bone microarchitecture (Boivin and Meunier, 2002) as well as bone mineralization (Rath et al., 2000). Bone mineralization is important for bone hardness and strength and involves a process where calcium phosphate crystals are produced and deposited within the bone matrix (Boivin and Meunier, 2002; Shim et al., 2012). Examining tibial ash, which is an important measure of bone mineral content (Kim et al., 2012), has been the most common method for assessing bone mineralization (Hall et al., 2003). Bone ash content is proportional to the degree of hardness of the bone (Bonser and Casinos, 2003) and the inorganic component of bone provides tensile strength and flexibility (Velleman, 2000) and the combination of these two components determine the breaking strength of bone (Rath et al., 1999). Increased mineralization and ash content result in increased bone breaking force (Talaty et al., 2009). Therefore, bone ash content can be used as an indicator of bone strength (Rath et al., 2000). Scott (2002), found that birds under 16L:8D had numerically higher toe ash content, compared to birds under 23L:1D. Lewis et al. (2009b) examined ash content and tibial breaking strength in broilers raised under 3, 6, 9, 12, 14, 16, 18, 20, or 22 h of darkness. Peak breaking strength was achieved when birds were provided 7.5 h of darkness, and the highest levels of ash occurred at 8 h of darkness. The authors suggested that the differences observed between lighting programs for breaking strength and ash content were influenced by level of activity, feeding behaviour, feed consumption, body weight and diurnal rhythm of hormones rather than a direct effect of light. However, lighting program affects each one of these factors so this statement may not be accurate. Bone density is determined by the mineral makeup of the bone matrix as well as the porosity of the matrix (Shim et al., 2012). Both factors influence the strength of the bone and therefore density can be an indirect indicator of strength (Shim et al., 2012). Onyango et al. (2003) found high correlations between percentage ash and bone mineral content (0.92) and bone mineral density (0.93). Therefore, these measures can be used as indicators of bone strength, however should not be relied upon to assess the walking ability of birds. Growth rate and body weight have the largest effect on walking ability (Sorensen et al., 1999; Kestin et al., 2001; Bizeray et al., 2002).

Brickett et al. (2007) demonstrated that providing longer periods of darkness resulted in higher bone mineral content, which is indicative of higher mineral density (Rath et al., 2000) as well as higher ash content. This could explain why Brickett et al. (2007) observed improved gait scores in birds under 12L:12D as compared to 20L:4D, though it should be noted that, in both lighting programs the gait scores were low with a mean of 1. Birds with gait scores of 3 or higher experience pain and therefore have compromised welfare (Danbury et al., 2000). Venalainen et al. (2006) found no obvious correlation between bone mineral content and the walking ability of broilers and Bizeray et al. (2002) also found that improvement in gait score was not associated with bone ash. Therefore, the improvement in gait score and thus walking ability could have been due to other beneficial effects of providing darkness. Some of these beneficial effects include an altered growth curve or an improvement in bone remodeling, as was previously mentioned. Another explanation is that birds raised with more darkness have increased overall activity levels, which is known to reduce leg problems (Reiter and Bessei, 1996). Bizeray et al. (2000; 2002) found that level of activity was inversely proportional to body weight and therefore an altered growth curve resulting in slower early growth can also result in higher bird activity, both of which improve skeletal health (Bizeray et al., 2000; 2002). However, Schwean-Lardner et al. (2012b) implemented periods of darkness and found that birds reared under 4D and 7D were heaviest, while birds reared under 7D and 10D had the highest activity. Schwean-Lardner et al. (2013) observed improved gait scores with longer periods of darkness. This study compared dark periods of 1D, 4D, 7D and 10D and found that using 1D resulted in approximately double the percentage of birds classified with gait scores of 3, 4, or 5 as compared to the other treatments. This effect also increased with age, likely due to increased body weight.

A common measure to assess skeletal development in poultry is to examine the occurrence and severity of skeletal disorders such as tibial dyschondroplasia (TD) and varus-valgus deformation (VVD). Tibial dyschondroplasia refers to a lesion characterized by abnormal cartilage accumulation in the legs of poultry (Leach and Nesheim, 1965; Leach and Mondonego-Ornan, 2007) and is often assessed using a qualitative scoring system. Sanotra et al. (2002) found that birds raised under continuous lighting had a higher occurrence and severity of TD than birds provided with a dark period. The authors also found that severity of TD was significantly correlated with impaired walking ability as measured by gait scores, therefore providing birds with access to a dark period significantly improved walking ability. While growth rate and feed intake are known to influence the development of leg abnormalities these factors were not measured during the study and may have explained why lighting program affected prevalence of

TD. However, other studies have shown that lighting program does not have an effect on TD (Onbasilar et al., 2008). VVD refers to an angular deformation of the tibitarsal bone (Randall and Mills, 1981; Julian, 1984). Valgus deformations refer to an outward rotation, while varus deformations are inward rotations (Gonzalez-Ceron et al., 2015). VVD is a multifactorial condition and a congenital defect may be a predisposing factor (Cruickshank and Sim, 1986), while high growth rate and low activity levels may worsen the condition (Riddell, 1983; Shim et al., 2012). Raising birds with longer periods of darkness results in both a slower early growth rate (Classen and Riddell, 1989) and an increase in overall activity (Schwean-Lardner et al., 2012), which suggests that it would also reduce the incidence of VVD.

1.3.2. Metabolic health

One of the largest impacts that providing darkness has on bird health is through reduction of metabolic diseases including sudden death syndrome (SDS) and ascites (accumulation of fluid in the abdominal cavity (Julian, 1993). Classen (2004) reported a reduction in SDS and ascites with longer dark periods, even in a genotype that has been genetically selected to have a lower incidence of metabolic disease. A study by Brickett et al. (2007) found the main cause of broiler flock mortality was SDS, which was effected by lighting program. Broilers under 20L:4D had a higher incidence of SDS, compared to those under 12L:12D. It should be noted that only these two lighting programs were compared and that the effects of a continuous or near-continuous lighting program or a moderate scotoperiod were not investigated. Lott et al. (1996) compared a 23:1D lighting program to an increasing program (12L from 3-21d, 14L at 21d and increased by one h each week until 23 h was reached at 41d) in two separate trials. The authors found that of the 10.5% total mortality, ascites accounted for 54% of that total mortality and birds raised on 23L:1D had a higher incidence than birds raised with increasing photoperiods. This was at least partially attributed to a decrease in early growth from 1-22d, whereas from 22-52d light restricted birds had the highest growth rate. Also, because little incidence of ascites was observed before 30 d, it suggests that development of ascites is effected by the timing that the rapid growth occurs and not solely by the occurrence of rapid growth.

1.3.3. Ocular development

Poultry eye development occurs in a diurnal pattern in response to changes in melatonin and dopamine production (Nickla, 2013), with growth occurring during light periods and ceasing during dark periods (Rada and Wiechmann, 2006; Egbuniwe and Ayo, 2016). When birds are raised under short dark periods, the diurnal rhythm is interrupted and therefore results in heavier eyes (Li et al., 1995; Lewis and Gous, 2009). Raising White Leghorn chicks and turkeys on continuous light or near-continuous light is known to cause eye abnormalities such as excessive eye growth, hyperopia, cataracts, and flattening of the cornea and lens (Li et al., 1995; Stone et al., 1995; Li and Howland, 2003; Leis et al., 2016), which have also been observed in chicks raised on 22L:2D and 23L:1D (Stone et al, 1995). This led Li et al. (2000) to evaluate the ocular development of chicks raised under continuous light compared to 1,2,3,4,6 or 12 h of continuous dark. The authors measured refraction, corneal curvature, anterior chamber depth, lens thickness, vitreous chamber depth and axial length. They found that providing a dark period of 4 h was the shortest period at which no negative effects on eye development were observed, which was later substantiated in broilers by Lewis and Gous (2009). The authors then wondered what the difference between a continuous 4 h dark period versus four 1 h dark periods spread out during the natural nighttime or equally distributed over a 24 h period would be. It was concluded that a continuous period of 4 h of darkness that occurs at the same time every 24 h was required for normal ocular development and growth.

While the above studies indicate that long photoperiods are detrimental to ocular development, the same can be said of long scotoperiods. Troilo and Wallman (1991) studied the effect of raising layer chicks from hatch until 4 wk under continuous darkness and found that birds eyes developed abnormally (myopia and hyperopia), however the conditions were reversible after a few weeks of exposure to a normal brooding lighting program of 14L:10D. Schwean-Lardner et al. (2013) studied the effect of increasing levels of dark exposure on eye weight in broilers and found that birds raised under 23L:1D had heavier eye weights than birds raised on 4, 7, or 10 h of darkness. These results are in agreement with previous studies conducted with layers that found 4 h of darkness promotes normal ocular development.

1.3.4. Immune function

Past research using a variety of species, including poultry, has shown that dark exposure improves immune function, through improved cellular and humoral immune responses (Kirby and Froman, 1991; Kliger et al., 2000; Moore and Siopes, 2000; Campo and Davila, 2002; Moore and Siopes, 2002; Abbas et al., 2008; Zheng et al., 2013). Zheng et al. (2013) found that including darkness in a broiler lighting program resulted in an improvement in antioxidant status as well as nonspecific immunity. Moore and Siopes (2000) observed a suppression of white blood cells in birds raised on continuous light, which suggests an impairment in their ability to mount a challenge to infection. Kirby and Froman (1991) observed a suppressed cellular immunity and secondary antibody response in young cockerels raised under continuous light compared to those raised under 12L:12D, most likely the result of increased synthesis of the neural hormone melatonin with increasing darkness.

Melatonin plays an important role in maintaining and regulating circadian rhythms in birds (Zeman et al., 1999). In poultry, various organs produce melatonin however, the primary site of production is in the pineal gland (Zeman et al., 2004). Production of melatonin follows a diurnal rhythm, with the peak occurring during the scotoperiod and the trough occuring during the photoperiod (Pang et al., 1996). If average production was significantly reduced it could result in desynchronization of these rhythms (Gwinner et al., 1997). Providing birds with a dark period results in a diurnal rhythm of melatonin production (Moore and Siopes, 2002) and longer dark periods result in increased peak production of melatonin (Zheng et al., 2013), as well as longer duration of melatonin release (Illnerova et al., 1984). Melatonin has both direct and indirect effects on immune function (Moore and Siopes, 2000; Moore and Siopes, 2002). Research has shown that it affects development of lymphoid organs (Moore and Siopes, 2002), stimulates leukocyte (Brennan et al., 2002) and lymphocyte (Kliger et al., 2000; Brennan et al., 2002) production, improves antibody formation (Maestroni et al., 1987), increases phagocytosis (Zheng et al., 2013), and initiates the secretion of cytokines (Garcia-Maurino et al., 1997). Melatonin also plays an important role in the antioxidant system through direct and indirect removal of free radicals and radical products (Tomas-Zapico and Coto-Montes, 2005). It has been suggested that broilers raised on continuous light are deficient in serum melatonin

(Apeldoorn et al., 1999; Schwean-Lardner et al., 2014). Kumar and Follett (1993) suggest that circulating levels of melatonin may be suppressed to basal levels in birds reared on constant light.

1.3.5 Stress

Dark exposure may also affect the stress response of broilers, which is often assessed using heterophil to lymphocyte (H:L) ratios (Campo and Davila, 2002). H:L ratios are a measure of long-term changes in the environment, which makes the measure a valuable tool for assessing environmental stress (Gross and Siegel, 1983). Many of the studies that have examined the effect of lighting program on the stress response were contradictory with regards to H:L ratios, but are difficult to compare because different lighting programs were examined. Campo et al. (2007) compared birds reared under a continuous lighting program with those raised under a 14L:10D program and found higher H:L ratios in birds exposed to continuous light. Similar results were obtained by Onbasilar et al. (2008) who compared the effects of rearing broilers under continuous light to 16L:8D. However, the study by Ozkan et al. (2006) showed no effect of lighting program (continuous versus 16L:8D) on H:L ratios. Moore and Siopes (2000) and Brennan et al. (2002) found that decreases in photoperiod length and supplementation of melatonin both resulted in reduced H:L ratios. Longer dark periods caused an increase in endogenous melatonin, thus resulting in lower H:L ratios. This implies that increasing levels of melatonin may reduce bird stress.

Assuming that fearful birds are also stressed birds, fear can also be used to measure stress. One test used to measure fear is the tonic immobility (TI) test, where birds are placed on their backs and timed until they right themselves back to their feet. The longer a bird takes to upright itself, the more fearful it is. Previous studies examining the effect of lighting program on TI have been contradictory (Sanotra et al., 2002; Campo et al., 2007; Onbasilar et al., 2008; Schwean-Lardner et al., 2012a) with some reporting no effect and others stating that continuous lighting resulted in longer periods of TI. Schwean-Lardner et al. (2012a) suggested that photoperiod had no relation to fear levels and instead the longer tonic state and the increased ease of catching birds raised on continuous or near-continuous light was possibly due to reduced mobility or reduced brain function of these birds. This reduced mobility and brain function may be attributed to sleep deprivation, rather than lameness which can affect TI (Vestergaard and Sanotra, 1999). Periods of light that are too long may result in higher stress due to sleep deprivation as well as lack of diurnal rhythmicity (Schwean-Lardner et al., 2014).

1.3.6. Mortality

Reducing mortality is important in terms of bird welfare, health and economics. Providing a dark period reduces the percentage of mortality that occurs in a flock. Classen (2004) saw a decrease in mortality as the length of the dark period increased from 4 to 12 h of darkness, with an intermediate effect occurring on 8 h of dark. Lewis et al. (2008) used dark periods of 8 and 16 h and found no difference in percentage of total mortality from 0-21 or 0-35 days of age. Therefore, it could be inferred that 8D is long enough to reduce mortality and increasing the duration of darkness beyond would result in no benefit. Schwean-Lardner et al. (2013) found that, depending on age, scotoperiod length had a significant linear or quadratic effect on causes of mortality, but in all cases, the highest mortality was found in flocks exposed to the shortest dark period (23L:1D). The same study also found differences in causes of mortality due to sex and genotype. Males were more susceptible to death from metabolic and skeletal diseases and had higher overall mortality than females. The study used birds from the 308 and 708 Ross genotypes and found higher overall mortality and mortality due to infections for all age groups in the 308 genotype. Possible reasons that increasing levels of darkness results in lower total mortality, especially mortality related to metabolic, skeletal and immune diseases, include a shift in the growth curve early in life, physiological changes via melatonin, an increase in bird activity and possibly improved sleep quantity and/or quality.

1.4 Experimental methodology

Observing and recording animal behaviour has become much more accurate and objective due to the development of new technologies (Dawkins, 2004). The use of video cameras allows for the collection of large amounts of data without disturbing the animal and infrared technology allows for accurate observation even during dark periods (Sergeant et al., 1998).

Two methods of observing animal behaviour include focal and scan sampling (Martin and Bateson, 1993). Scan sampling refers to observation of a group of individuals at reoccurring time intervals over a specified period, with the behaviour of each bird recorded at that instant and then expressed as a percentage of birds (translating into percentage of time) performing each activity over the entire period. This allows for analysis of flock rhythms and overall behavioural activity, but does not allow observation of individual behavioural patterns (Altmann, 1974). With focal sampling, a predetermined number of individual birds are randomly selected and individually marked for observation. Those individual birds are observed for an entire time period in order to accurately determine individual behavioural patterns, such as feeding and drinking behaviours.

When analyzing short-term feeding behaviour it is essential to properly define bout criteria. One bout can include several visits to the feeder separated by short time intervals (time between feeding events), hence using individual visits to feeders may lead to inaccurate conclusions (Tolkamp and Kyriazakis, 1999). Instead, grouping visits into bouts, which is a more biologically relevant unit of feeding behaviour, allows for more accurate interpretation of results (Tolkamp et al., 1998, 2000). Therefore, in order to accurately analyze bout patterns, the correct bout criterion must be determined, that is, the shortest time interval that defines the separation of one bout from another (Tolkamp and Kyriazakis, 1999). Grouping feeding events into bouts can allow for the comparison of feeding behaviour between different species as well as using the same method to estimate meal criterion (Howie et at., 2010; Tolkamp et al., 2011).

1.5 Impact of dark exposure on behaviour

1.5.1. Sleep

While it is known that sleep is important, the causation and function are not well known for poultry (Blokhuis, 1983, 1984; Malleau et al., 2007). In terms of physiology and behaviour, sleep is relatively comparable between poultry and mammals (Blokhuis, 1983). It has been suggested that the primary functions of sleep are energy conservation, tissue regeneration, growth, and brain function (Zepelin and Rechtschaffen, 1974; Rechtschaffen et al., 1983; Malleau et al., 2007). During rest/sleep biosynthetic pathways may be activated due to energy conservation, thus promoting anabolic processes in tissue (Adam, 1980). It has been shown in rats that protein synthesis rates were highest during the rest/sleep period (Rebolledo and Gagliardino, 1971; Rau and Meyer, 1975). Greater protein synthesis may be linked to increased mitosis, which is important for tissue maintenance and the highest mitotic rates occur during the rest/sleep period (Adam, 1980). Increases in the rate of mitosis, protein synthesis and energy conservation during rest/sleep may be due to an increase in the release of growth hormone (Korner, 1965; Rudman et al., 1973).

There are different types of sleep, including active and quiet sleep, both of which are necessary for proper body function (Ookawa and Gotoh, 1964; Blokhuis, 1984). Active sleep is characterized by low-amplitude, high-frequency encephalogram (EEG) activity, whereas quiet sleep results in low-frequency, high-amplitude activity (Rattenborg et al., 2000). In chickens, the active phase may indicate a state of deeper sleep than the quiet phase (Ookawa and Gotoh, 1964). A study by Ookawa and Gotoh (1964) showed that birds resting during the light period had EEG patterns indicating quiet sleep and during the dark period EEG patterns of both quiet and active sleep were observed. Therefore, the quality of sleep is improved by providing a dark period. Use of continuous or near-continuous lighting programs likely result in birds being disrupted when they attempt to sleep or rest, due to pen mates performing other activities (Malleau et al., 2007). Birds raised with less than four h of darkness lack a synchronized flock behavioural rhythm and circadian melatonin rhythm, which results in sleep fragmentation (Schwean-Lardner et al., 2014). Sleep fragmentation is a type of sleep deprivation that occurs when birds experience repeated disruptions, which cause awakenings (Bonnet, 2005). Therefore, sleep deprivation may be due to poor quality or quantity of sleep (Chen and Kushida, 2005).

A study by Malleau et al. (2007) found that rest is important to birds, especially during the first 3-5 d of life, which is interesting because during this period continuous or near-continuous light is often provided to help birds locate the feeders and drinkers. A study providing 8D and 16D at 2 d of age showed that chicks were able to locate feeders and drinkers during dark periods with no detrimental effect on body weight or feed intake (Lewis et al., 2008).

1.5.2. Diurnal rhythms

In birds, one of the external factors that stimulate physiological/behavioural rhythms is the provision of regular light and dark periods (Yang et al., 2015). Dark exposure results in an increase in melatonin production, resulting in higher levels during the scotoperiod and lower levels during the photoperiod as was previously discussed. Melatonin is important in establishing diurnal rhythms relating to body temperature, secretion of immune cells and metabolic functions that influence feed and water intake as well as digestion (Binkley et al., 1973; Bernard et al., 1997, Apeldoorn et al., 1999). Sanotra et al. (2002) suggested that a lighting program with adequate darkness would allow birds to maintain these diurnal rhythms, allowing them to organize patterns of behaviour, including feeding. The majority of feeding takes place during the photoperiod, with minimal to no feeding occurring during the scotoperiod, depending on the duration of light:dark provided. In a review by Savory (1980) it was noted that during the photoperiod broilers ate the most at the start and end of the photoperiods, ranging from 8L to 16L.

1.5.3. Feeding behaviour

The use of lighting programs has also been shown to have an effect on bird behaviour (Savory 1976; Duve et al., 2011; Schwean-Lardner et al. 2012a), however producers do not usually take into account behaviour when choosing a lighting program instead focusing on production variables (Mauldin and Graves, 1984). Most of the work that has examined the behaviour of broilers raised on different lighting programs has done so for only a portion of the light period and/or used scan sampling (Sanotra et al., 2002; Lewis and Morris, 2006; Lewis et al., 2009a; Schwean-Lardner et al., 2012a, b, 2014). This would have allowed researchers to infer what proportion of the day birds spent feeding, but would not allow for the analysis of individual feeding behaviour. Feeding behaviour is a multifaceted concept, involving many different aspects, but commonly used measurements are those describing patterns of feed intake, which in the short-term include meal size, duration and frequency (Nielsen, 1999). These patterns of feed intake are measured on an individual basis whereas feed intake and total time spent feeding are measured daily. Because poultry have a 24 h circadian rhythm, the use of daily means and measures is justified (Nielsen, 1999). Previous research has shown that feeding behaviour is effected by daylength (Savory, 1976; May and Lott, 1992; Buyse et al., 1993; Duve et al., 2011; Schwean-Lardner et al., 2012a, 2014), however the effect on specific feeding behaviour components including the number and length of feeding bouts, and the pattern of feeding over a 24 h period is still unknown to the author's knowledge. While the effect of varying dark exposure durations is still undetermined, Bokkers and Koene (2003) found an age effect with older birds spending less time feeding, having fewer meals per hour, consuming more feed per hour and having longer meal and interval lengths. The authors suggested the reason for this was because of the increase in body size as the birds approached market age.

Lighting programs may simulate "dawn" and "dusk" in order to prepare birds for the beginning of light and dark periods in a more "natural" fashion (Prescott et al. 2003). Including simulated dawn and dusk periods in a lighting program has resulted in slightly improved growth, due to higher feed intake and increased feed efficiency (Savory, 1976). Lewis and Morris (2006) suggested that providing a dusk period allows broilers to better predict the end of the photoperiod and facilitates an increase in feeding activity to ensure a full crop at the beginning of the scotoperiod. May and Lott (1992) reported that birds given a dark period learn to anticipate feed withdrawal and compensate by increasing feeding activity before the scotoperiod begins.

Internal control mechanisms for feed intake are externally expressed as feeding behaviour characteristics including meal size, duration, frequency and time of occurrence. These factors can be used to study the regulation of feed intake (Reddingius, 1980; Bokkers and Koene, 2003). Bokkers and Koene (2003) studied the correlations between meal length and interval length. Correlations between the length of a meal and the length of the following interval before the next meal could suggest that interval length may be regulated by a hunger mechanism (Savory, 1981). Correlations between the length of an interval and the next meal length could suggest that meal size may be regulated by a satiety mechanism (Savory, 1981). The results indicated that feeding behaviour in broilers was controlled by satiety mechanisms rather than hunger mechanisms. The regulation of feed intake in poultry involves peripheral tissue and central nervous system signaling pathways that are regulated by hormonal, neural, neuroendocrine, and nutrient signaling mechanisms (Richards and Proszkowiec-Weglarz, 2007). Savory (1981) also suggested that broilers, as compared to layers, voluntarily consume amounts of feed that approach their maximal gastrointestinal tract (GIT) capacity. These results are in agreement with earlier work by Nir et al. (1978) who looked at the effect of overfeeding on genetic lines selected for low and high body weights. The results of the study showed that broilers selected for high body weights could only be force-fed 13% more than their ad libitum consumption, compared to low body weight strain birds who were overfed up to 70% more. This indicates that selection for increased body weight likely effects the feeding behaviour of these birds, who may be more motivated to feed, which could be reflected in an increase in feed consumption and therefore body weight. In the decades since that study was performed, birds have continued to be selected for higher growth rates, suggesting that modern birds also feed to levels approaching their full GIT capacity. Barbato et al. (1984) suggested that the limit set by the size of the GIT could result in a future plateau of the selection response for increased body weight. However, there is no evidence that this plateau has been reached at this time.

1.5.4. Impact of genetic selection on feeding behaviour

Broiler chickens have been genetically selected for increased growth rate and more efficient feed conversion, which is associated with changes in feed intake and behaviour (Weeks et al. 2000). However, Howie et al. (2009) found no evidence that selection for increased growth had altered the structure of feeding behaviour. This was supported by Howie et al. (2011) who found low genetic correlations between performance and feeding behaviour traits, which indicates that the difference in selection intensity for production traits among lines has had a limited effect on feeding behaviour.

Even in birds within the same genetic line, there can be different feeding strategies to achieve the same feed intake. This variation in feeding behaviour could be useful in selecting birds able to resist certain environmental pressures (Howie et al., 2011). The authors provided the following examples to highlight certain environments that would benefit from selection for different feeding strategies: (1) where feed was not readily available, birds that consume larger, less frequent meals could benefit; (2) when feed was readily available, but of poor quality, birds that consume smaller, more frequent meals could perform better; and (3) when feed competition is high or variation in BW occurs, it could be beneficial to select birds who feed throughout the day. However, it seems as though birds are able to quickly and successfully adapt their feeding behaviour to accommodate different environments. For example, birds learn to anticipate dark periods and will eat more before and after the dark period or will learn to feed during the dark to compensate (May and Lott, 1992; Lewis et al., 2008; Duve et al., 2011).

1.5.5. Impact of dark exposure on feeding activity

In terms of overall activity, birds raised under continuous or near continuous light are less active than birds who are provided a dark period of at least 4 h (Schwean-Lardner et al. 2012a). A possible explanation for this is that the birds reared under continuous or near-continuous light are less mobile, indicated by the poorer mobility (lower gait scores) observed by Sanotra et al. (2002), Ozkan et al. (2006), and Schwean-Lardner et al. (2013). This effect of dark exposure on overall bird activity also impacts feeding activity. Duve et al. (2011) examined the effects of a continuous 8 h dark period (Dark 8) versus two 4 h dark periods (Dark 4+4) on feeding behaviour. The authors found that birds on both lighting programs reacted to the beginning of the photoperiod by increasing their feeding behaviour, with the Dark 4+4 program showing a higher proportion of birds feeding in the 20 minutes immediately following the beginning of the photoperiod. While the Dark 8 birds did not display as large of an increase in feeding behaviour during this time, they displayed higher overall feeding activity over the 24 h period. Feeding activity plateaued thirty minutes after the photoperiod began, with Dark 8 birds feeding for a longer period than Dark 4+4 birds. This increase in feeding immediately after the end of the scotoperiod could be due to hunger (Scanes et al., 1987). Dark 8 birds also demonstrated an increase in feeding activity 3 h before the scotoperiod began, but Dark 4+4 did not exhibit this behaviour. The Dark 4+4 birds did not anticipate darkness by increasing their intake and the authors suggested this may have been because feed remaining in the digestive tract was sufficient to last the shorter scotoperiod. Another suggestion was that these birds were unable to predict the scotoperiod, but because no change in feeding activity was seen before either dark period the prior suggestion seems more likely. Schwean-Lardner et al. (2014) did not observe any anticipatory feeding behaviour in birds raised on 20L:4D, which suggests that birds require dark periods of >4 h in order to be able to anticipate them (Classen et al., 2016). Interestingly, Duve et al. (2011) found that fewer Dark 8 birds fed during the feeding peak that occurred after the scotoperiod, whereas it was expected that birds on the longer scotoperiod would have a higher motivation to feed due to hunger. Scanes et al. (1987) suggested that the increase in feeding activity observed at the beginning of the photoperiod was due to hunger and that when no increase was observed, this was due to feed being stored and available for digestion during the dark period. Therefore, it may be because the Dark 8 birds anticipated the dark period and filled their crops prior to the scotoperiod beginning that less of these birds visited the feeder after the end of scotoperiod. It was hypothesized that feed may have been digested at a slower rate to maintain a source of energy throughout the dark period, allowing birds to avoid entering a state of hunger (Duve et al., 2011).

Buyse et al. (1993) examined the feeding activity of broilers raised on 14L:10D, during the photoperiod and scotoperiod. This study differed from Duve et al. (2011), in that 10 h of continuous darkness instead of 8 h was used. Results were similar between studies, in that there was little feeding activity during the scotoperiod and peak activity occurred at the beginning of the photoperiod and prior to the scotoperiod which suggested anticipation of the dark period.

Studies have shown that the majority of feeding behaviour is performed during the photoperiod, with little to no activity during the scotoperiod unless given a relatively long dark period (Savory, 1976; Lewis et al., 2009a; Duve et al., 2011; Tolkamp et al., 2011). Schwean-Lardner et al. (2012a) conducted an experiment to examine the effects of graded increments of darkness on broiler behaviour, during both the photoperiod and scotoperiod for an overall period of 24 h. The authors' hypothesis was that the addition of darkness to a lighting program would result in increased behavioural expression, including increased feeding behaviours. The study found that, during the photoperiod, birds raised on a 23L:1D program spent the least amount of time (%) at the feeder at both 28 d (7.61%) and 43 d (5.16%) of age. The birds reared under 17L:7D spent the most time at the feeder at 28 d (12.87%). However, at 43 d, broilers reared under 14L:10D spent the most time at the feeder (11.82%). Analysis of the production data by Schwean-Lardner et al. (2012b) showed that at 32 and 39 d of age the lowest body weights were found under the 14L:10D treatment, however at 49 d there was no difference in BW between birds raised on 14L:10D or 23L:1D. The 23L:1D birds ate more than birds under 14L:10D at each age and walked the least, which suggested that they ate less frequently and had larger meals. The authors also observed that birds under 10D were the only birds who visited the feeder during the scotoperiod, which may have been because they experienced a state of hunger during the dark period and/or they had received enough rest and were active and exhibiting behaviours usually seen during the photoperiod (Schwean-Lardner, 2012a).

1.5.6. Relationship between dark exposure and feed passage time

The crop is often thought of as primarily a storage compartment, however its structure and anatomy suggest that it could interact with other segments of the GIT and play a role in digestion and GIT health (Classen et al., 2016). Enzymes from feed, water, saliva and microorganisms are capable of initiating digestion and the extent is dependent on the amount of feed that enters the crop and the amount of time it remains there (Classen et al., 2016). A continuous lighting program combined with ad libitum feeding makes it unnecessary for birds to use the crop for storage and therefore feed passes quickly from the crop into the gizzard (Chaplin et al., 1992). Shires et al. (1987) reported that broilers raised on continuous lighting had a crop retention time of 7.4 minutes. However, when birds are provided with a period of darkness, a drastic change in crop utilization occurs. Cutler et al. (2005) found that in turkeys raised on 14L:10D, the crop retention time was 9 h after the beginning of the scotophase. This suggests that birds can retain feed in their digestive tracts for the entire dark period, due to anticipatory feeding behaviour and an increase in the amount of feed stored in the crop as well as the time it remains there. Regulation of crop emptying is largely controlled by the gizzard (Chaplin et al., 1992; Jackson and Duke, 1995), which controls the rate of feed passage for the GIT (Svihus, 2011; Classen et al., 2016).

Duve et al. (2011) examined the effects of a continuous 8 h dark period (Dark 8) versus two 4 h dark periods (Dark 4+4) on digestive transit time in broilers. Data collected from excretion curves and retention times indicated that, during the dark period, feed remained in the digestive tract for longer and was released more slowly in birds exposed to a longer scotoperiod, in order to compensate for the energy demands of a longer period without feed (Duve et al., 2011). Previous studies have also found that feed transit time was significantly longer during the scotoperiod for broilers (Buyse et al., 1993) as well as turkeys (Cutler et al., 2005) each raised on 14L:10D. It has been estimated that the retention time in the gastrointestinal tract, excluding the ceca, is 4 to 8 h for broilers raised under continuous lighting (Hetland and Svihus, 2001). However, when birds are raised with a period of darkness, this retention time becomes increasingly longer with longer scotoperiods. This increased retention time results in digestion and fermentation occurring in the crop (Cutler et al., 2005). It is also possible that better digestion occurs throughout the GIT, which could be another mechanism by which feed efficiency is improved.

1.5.7. Relationship between dark exposure and digestive tract segment and content weights

Evidence provided in the above section proves that dark periods result in a slower feed transit time and this in turn may result in a change in the full and empty weights of the segments of the GIT. Changes in feed intake will also affect the content weights. Duve et al. (2011) examined the effects of a continuous 8 h dark period (Dark 8) versus two 4 h dark periods (Dark 4+4) on the weight of intestinal segments and contents in broilers. Relative crop content of Dark 8 birds was significantly higher prior to the scotoperiod at all ages, indicating that the observed increase in feeding activity was due to anticipation of the dark period and not hunger. Buyse et al. (1993) found that during the photoperiod, the crop and proventriculus/gizzard only contained a small amount of ingesta. The amount then increased 10.5 fold for the crop and 2.8 fold for the proventriculus/gizzard at the beginning of the scotoperiod before gradually decreasing. The authors estimated that the storage of ingesta and the increased retention and transit time during the scotoperiod provided 75.5% of the nightly energy requirement (Buyse et al. 1993). This illustrates that broilers, using their crops for storage, can retain feed in their digestive tracts for the majority of the dark period, thus possibly reducing the time they are feed deprived and experiencing a state of hunger. Jackson and Duke (1995) also found that storage of feed in the crop allowed turkeys to maintain a supply of feed to the rest of the GIT during the dark period.

Warriss et al. (2004) measured GIT content weights after a feed withdrawal period of 2,4,8,12,18 or 24 h in broilers reared on continuous light. The authors found that feed deprivation had the greatest effect within the first 8 to 12 h of withdrawal. After 8 h the total weight of the

GIT contents in feed deprived birds was 35% less than that of ad libitum fed birds. However, GIT clearance and motility is complex and effected by many factors, including feed withdrawal and pattern of intake prior to withdrawal, temperature, and bird activity level (Duke et al., 1997), which makes comparison of trials with different housing and rearing conditions difficult. The largest decrease in weight occurred in the crops of fasted birds. This is unsurprising because birds reared under constant light often do not use their crops for storage and therefore, feed transit time is fast.

1.5.8. Drinking behaviour

Drinking behaviour can often be linked to feeding behaviour. Schwean-Lardner et al. (2014) found that drinking patterns were similar to feeding patterns in that peaks occurred at the beginning and end of the photoperiod. Symeon et al. (2010) found that the probability of a bird feeding was significantly and highly correlated with the probability of a bird drinking. The authors fed broilers oregano supplemented diets and observed a decrease in the number of visits to both the feeder and drinker as a result of the supplementation. The essential oil was only added to the diet, not the watering system, therefore the decrease in drinking observed was not due to a novel/aversive taste or smell of the water. Rather, the decrease in drinking may have occurred due to a reduction in feeding, which was related to the aversive taste and/or smell of the essential oil. Warriss et al. (2004) performed an experiment looking at the effect of feed and water withdrawal on defecation and weight of GIT contents. After 4 h of feed deprivation, a decrease in drinking was observed and after 24 h water consumption decreased to nearly half of the consumption of birds given feed and water ad libitum. The authors suggested that the correlation between feeding and drinking was evidence that dry feed intake stimulates drinking.

Monitoring drinking behaviour can help identify different drinking strategies birds utilize. Drinking behaviour is effected by bird age, with longer and fewer bouts occurring as birds get older (Ross and Hurnik, 1983). Again, as with feeding behaviour, visits to the drinker are clustered together into bouts through use of a bout criterion. Rusakovica et al. (2015) analyzed drinking behaviour traits in two lines of turkeys. The authors studied a male line (6-9 wk of age) and a female line (10-13 wk of age). An electronic water station was used to record individual bird drinking behaviour and video observation was used to correlate water intake records and individual drinking behaviour. The results demonstrated that birds from the male line had longer but less frequent visits to the water station, whereas birds from the female line showed more variation in the time between visits. The authors also found the probability of a bird re-visiting a drinker within five minutes of the previous visit was different for visits during the light and dark periods in both lines. This suggests that turkeys organize their drinking behaviour differently during the light and dark periods. Critical review of this work was not possible as only the abstract was available for review therefore no materials and methods or results section was available.

1.5.9. Impact of dark exposure on bird welfare

The presence of regular light and dark periods, which are of adequate length to stimulate diurnal rhythms, impacts broiler welfare (Ozkan et al. 2006). Birds raised on longer daylengths are more inactive, spending less time performing behaviours relating to mobility, nutrition and comfort (Schwean-Lardner et al., 2012a). Referring back to the Five Freedoms, particularly the freedom to express normal behaviour, it is clear that using continuous or near-continuous lighting negatively impacts animal welfare. The reduction in most of the behaviours observed in the study by Schwean-Lardner et al. (2012a) indicated that long daylengths not only had a negative effect on bird welfare but may also negatively impact bird health and productivity, which was later substantiated (Schwean-Lardner et al. 2012b, 2013). Schwean-Lardner et al. (2012a) suggested that birds on longer daylengths ate less frequent and larger meals, which may have been due to a reluctance to move due to lethargy from sleep deprivation or leg abnormalities. Longer daylengths increase the occurrence of leg abnormalities, whereas periods of darkness allow birds to rest, which has been shown to have a positive effect on skeletal growth (Classen and Riddell, 1989; Classen et at, 1991; Sanotra et al., 2002; Brickett et al., 2007; Schwean-Lardner et al., 2013). Longer daylengths may also cause a reduction in mobility related behaviours, which could increase the occurrence of breast blisters, hock burns or foot pad lesions, due to increased time spent resting (Gordon, 1994). The above-mentioned issues all reduce the welfare of the birds and may result in increased mortality due to culling and/or increased condemnations at the packing plant.

1.6 Conclusions

The information presently available in the literature suggests that the use of constant or near-constant light results in poorer bird performance, health, and welfare. Measuring the feeding and drinking traits of individual birds can increase our knowledge of how group-housed birds organize their feeding and drinking strategies. In doing so, the results of this work may provide a better understanding of how birds adjust to different periods of darkness and how changes in feeding behaviour alter the passage of feed through the gastrointestinal tract. Identification of different individual feeding and drinking strategies could also be useful in the future for selection criteria in breeding programs.

To conclude, there is evidence that varying the length of darkness that birds are exposed to has an impact on their feeding behaviour. The majority of past work in the area often involved the use of one lighting program as compared to another lighting program, without looking at the relationship of the lighting program to the variable being studied.

1.7 Objectives

The primary objective of this study was:

- to determine the effect of dark exposure on the feeding behaviour of male and female broiler chickens at weeks 2 and 4 of the production cycle.
    - The parameters assessed include number, duration, frequency, and pattern of feeding bouts over a 24 h period.

A second objective was:

- to investigate the effect of dark exposure on broiler productivity from 0-31 d and gastrointestinal tract segment and content weights at 27-28 d of age.

    - The production parameters assessed were growth rate, feed intake, feed efficiency, and mortality. Gastrointestinal tract segment and content weights were calculated by recording the full and empty weights of the GIT segments over a 24h period.

1.8 Hypotheses

The hypotheses of the study include:

- Duration of darkness will affect feeding behaviour.
    - Broilers exposed to longer dark periods will have more frequent, but shorter feed bouts, because birds exposed to longer dark periods are more active and will therefore visit the feeder more frequently.
    - Birds reared on >1 h of darkness will anticipate when lights turn off and consume more feed prior to in order to retain a source of feed for the majority of the dark period.
- Longer dark periods will result in a slower feed passage rate, as indicated by the content weight of different segments of the gastrointestinal tract.
- Feeding behaviour will differ with age and between genders.
    - As birds age, they will not visit the feeder as often but the duration and interval between their visits will be longer due to heavier body weight, increased gut capacity and a reduction in activity.
    - Males will consume feed more often than females due to their larger body size and higher feed requirement.

2.0 Chapter 2: Effect of dark exposure on production parameters and gastrointestinal tract segment and content weights in commercial broilers

The objectives of this work were to examine the impact of darkness duration on the feeding behaviour, productivity, and gastrointestinal tract segment and content weights of broilers. The data in Chapter 2 focused on production data, including growth, feed intake, feed efficiency, and mortality and how these parameters were effected by darkness, gender, and bird age. The GIT data, comprised of the segment and content weights, were studied to determine the impact of dark exposure and time of day.

2.1 Abstract

The impact of darkness on productivity of Ross 308 broilers (7-31 d) and gastrointestinal tract segment and content weights (27-28 d males) was examined, with dark exposure (23 h light:1 h dark (1D), 4D, 7D and 10D) and gender as independent variables. Birds (n=4000) were placed in 8 identical rooms with 8 pens per room (2 replications per lighting treatment and 4 replications per gender per room) at day of age. Pen body weight (BW) were collected at 0, 7, 21, and 31d and feed intake and efficiency were calculated for each period and overall. At 27d, 6 birds per lighting program were euthanized at 2h intervals over 24h. Bird weight and weights of the full and empty crop, proventriculus, gizzard, duodenum, jejunum, ileum and ceca were recorded. Production data were analyzed in a 4 (dark exposure) x 2 (gender) x 2 (age) factorial arrangement and GIT data were analyzed in a 4 (dark exposure) x 12 (time of day) factorial arrangement, both with lighting program nested within room, using Proc Mixed of SAS. Dark exposure data were also analyzed using regression analyses. Differences were significant when $P \leq 0.05$. At 31d, regression analysis did not show an effect on BW, however numerically birds raised with 4D and 7D were heavier (2.12 and 2.14kg, respectively) than 1D or 10D birds (2.09 and 2.08kg, respectively). Feed consumption showed a quadratic response with maximum intake under 4D. Birds provided 10D (0-31d) were the most feed efficient (linear response). Regression analyses showed a quadratic effect on mortality, with the highest mortalities under 4D and 7D. Dark exposure and full crop weight were related in a quadratic fashion, with heaviest full crops found on 10D (26.3g) and lightest on 1D (12.8g). The relationship between empty crop weight (linear) and empty crop weight (% of BW) (quadratic) and darkness indicated that birds on 10D had the heaviest crops (5.8g, 0.3% of BW). The crop content (% BW) changed throughout the day (quadratic) for all treatments except 1D. The empty gizzard weight (% BW) increased linearly as darkness increased, while empty duodenum and jejunum weight (% BW) decreased linearly with increasing darkness. In conclusion, darkness has a positive effect on body weight and feed efficiency. The crop data suggests that birds can anticipate dark periods of 4 h or more and fill their crops prior to darkness. Birds raised on longer dark periods had larger gizzards and smaller intestines, possibly suggesting improved digestion or feed conversion.

Key words darkness, productivity, gastrointestinal tract weight, gastrointestinal content, broilers

2.2. Introduction

Research indicates that providing birds with moderate periods of darkness results in numerous production, health and welfare benefits (Schwean-Lardner et al., 2012a, b, 2013, 2014). In terms of production benefits, providing darkness results in improved feed efficiency and lower bird mortality (Classen, 2004; Lewis et al., 2008; Schwean-Lardner et al., 2012b, 2013). The influence that providing darkness has on body weight has been variable between studies. Ingram et al. (2000) looked at BW in broilers from 0-2, 0-4, and 0-6 wk and found that, at each period, birds reared on 23L:1D weighed more than those reared on 12L:12D. It is possible that neither of the two scotoperiods compared in the study were optimal, whereas a more moderate dark period could be beneficial. A later study by Onbasilar et al. (2008) found no difference in final BW at 42 d between broilers raised on 24:0D or 16L:8D. Schwean-Lardner et al. (2012b) reared broilers under 23L:1D, 20L:4D, 17L:7D or 14L:10D to 32, 39, or 49 d of age and assessed their productivity. The authors found that broiler body weights were heaviest in birds raised under 20L:4D at both 32 and 39 d. At 49 d, body weights under both 17L:7D and 20L:4D were significantly heavier than under 14L:10D and 23L:1D. This suggests that growth data, particularly under longer dark periods, may be age dependent.

As for health benefits, a lower incidence of skeletal abnormalities (Classen and Riddell 1989; Classen and Riddell, 1990; Sanotra et al., 2002; Schwean-Lardner et al., 2013) and metabolic diseases (Classen, 2004; Brickett et al., 2007; Schwean-Lardner et al., 2013) have also been recorded in birds exposed to at least 4 h of dark. One of the mechanisms for this improvement in health appears to involve relative growth rate at various ages. Exposing birds to a diurnal pattern with an adequate dark period early in life results in slower early growth, followed by compensatory gain later in life (Classen, 2004; Bayram and Ozkan, 2010; Schwean-Lardner et al., 2012b). This shift in the growth curve is likely one of the reasons for the improvement in skeletal quality, as slower growth allows for enhanced skeletal system development (Robinson et al., 1992; Sanotra et al., 2002; Schwean-Lardner et al., 2012b). Slower early growth also plays a role in reducing the occurrence of metabolic diseases, such as ascites and sudden death syndrome (Classen et al. 2004; Brickett et al., 2007; Schwean-Lardner et al., 2013), likely by improving the development of the cardiac and respiratory systems. While rapid growth rate does play a role in increased mortality (Robinson et al., 1992), especially in terms of skeletal and metabolic disease, lack of darkness itself also has an effect (Schwean-Lardner et al., 2013).

Another mechanism for how darkness improves production variables is that providing a continuous dark period, of adequate length, allows birds a time to sleep/rest uninterrupted (Brickett et al., 2007; Schwean-Lardner et al., 2012a, 2014). It has been hypothesized that lighting programs which use continuous or near-continuous lighting result in birds often being interrupted when they attempt to sleep/rest by conspecifics (Schwean-Lardner et al., 2012a, 2014). Sleep, although not well understood in poultry, is believed to be important for growth, energy conservation, tissue repair, and brain function (Adam, 1980). A review of the subject eludes that the optimal time for protein synthesis, important for growth and regeneration of brain and body tissues, would be during rest/sleep after a period of feeding (Adam, 1980). Darkness also results in an improvement in immune function (Kirby and Froman, 1991). Changes associated with each of these factors may at least partially explain the lower mortality rates that have been observed on longer dark periods.

Broilers raised with dark periods of adequate length (>4 h) are able to anticipate these scotoperiods (Schwean-Lardner et al., 2014; Classen et al., 2016) and will increase their feeding activity prior to darkness in order to keep feed in their digestive tract for the majority of the dark period (Duve et al., 2011). To achieve this, crops are utilized for storage and feed passage rates are decreased (Buyse et al., 1993; Duve et al., 2011). Feed passage rate can affect bird performance and GIT health as well as nutrient digestibility (Svihus et al., 2002). A slower feed passage rate allows for improved nutrient absorption and utilization (Latshaw, 2008), which results in more efficient use of nutrients from the diet and may improve growth performance (Poorghasemi et al, 2013). Feed passage may also influence the microbiota populations in the GIT, which could affect nutrient digestion (Choct et al., 1996) as well as gut health. A slower feed passage rate results in longer digesta retention time which supports bacterial colonization and activity in the small intestine (Waldenstedt et al., 2000). This would only be advantageous if colonization of beneficial bacterial species were promoted. The maximum GIT fill capacity is limited by feed passage rate and digesta volume (Svihus et al., 2002).

The objective of this chapter was to investigate the effect of dark exposure on broiler productivity and GIT segment and content weights. The production parameters assessed were growth rate, feed intake, feed efficiency, and mortality. GIT segment and content weights were calculated by recording the full and empty weights of the GIT segments over a 24 h period. It was hypothesized that use of dark periods in a broiler lighting program would improve bird productivity and alter GIT segment and content weights. Also, longer dark periods would result in a slower feed passage rate, as indicated by the content weights of different segments of the GIT.

2.3 Materials and Methods

2.3.1. Experiment

The experimental protocol for this trial was approved by the University of Saskatchewan Animal Care Committee and was performed under the recommendations of the Canadian Council of Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

A trial was conducted to examine the effect of dark exposure, gender, age, and their interactions on production parameters in broiler chickens over a 31 d period. The experiment included a total of 4,000 Ross x Ross 308 male and female broilers and consisted of two room replications of each of the four lighting programs.

During this trial, GIT segment full and empty weights were also collected to study the effect of dark exposure on GIT segment and content weights, and therefore, indirectly, on feed passage time.

2.3.2. Housing and management

At the time of placement (d 0) 1,888 male and 2,112 female Ross x Ross 308 broilers were randomly distributed among eight identical rooms (12.19m x 7.01m) upon arrival at the University of Saskatchewan Poultry Centre, and were reared until 31 d of age. Each room was separated into 8 pens (2.3m x 2.0m) with 4 pens assigned to males and 4 to females. Pens were stocked at an estimated final density of $32 kg/m^2$ (66 females per pen; 59 males per pen) based on 32 d weights listed under the Performance Objectives for Ross 308 birds (Aviagen, 2014). From placement to d 7, all birds were maintained on 1D and on d 7, lighting treatments were initiated. The lighting programs used were as follows: 14L:10D (10D), 17L:7D (7D), 20L:4D (4D) and 23L:1D (1D), with darkness provided in one continuous period. Lights turned on at 06:00 for all lighting treatments. Lights turned off at 20:00 (10D), 23:00 (7D), 02:00 (4D) and 05:00 (1D). Light intensity was the same in each room (25 lux to d 7, then 5 lux for the remainder of the trial), with light provided by incandescent bulbs. Light intensity was 0 lux during the dark period. Dawn and dusk were simulated in all rooms and were included in the photoperiod, with a 15 minute duration for both. The room temperature curve started at 33°C on day 0 and was gradually reduced to 21°C by 31 d. Feed (crumble/pellet form) was provided ad libitum in one tube feeder per pen (circumference of 112 cm) and water via Lubing nipple drinkers (Lubing Systems LP, Cleveland, TN, USA; six nipples per pen) for the duration of the trial. Birds were fed 0.65 kg of a commercial starter ration per bird and the balance of feed until the end of the trial was a commercial grower ration (Table 2.1). Litter material was wheat straw and was used for the duration of the trial.

2.4 Data collection

2.4.1. Production data

At 0, 7, 21, and 31 d of age, birds were counted and weighed (on a pen basis) and remaining feed was weighed to allow for the calculation of individual average bird weight, feed intake, and feed efficiency. Birds were examined daily, and if cull birds were identified, they were humanely euthanized via manual cervical dislocation, and their body weight was recorded. All mortalities were collected, weighed and recorded on a daily basis.

2.4.2. Gastrointestinal tract segment and content data

On d 27 and d 28 (24 h period), three male birds were randomly selected at each time period (08:00, 10:00, 12:00, 14:00, 16:00, 18:00, 20:00, 22:00, 24:00, 02:00, 04:00, and 06:00) from one predetermined pen in each room for collection of full and empty GIT segment weights, which allowed for the calculation of content weights (wet basis). A total of 6 birds per lighting program per time period, for a total of 288 birds, were used for this data collection. Birds were euthanized with a T-61 solution (Hoechst Roussel Vet, Regina, SK) injected into the brachial vein at a concentration of 0.3mL/kg. Each bird was given a number (1, 2, 3), weighed and the following data were recorded: room, pen, time, bird number, and body weight. Full GIT segment weights were recorded. Contents were then removed and empty segments were cleaned and patted dry before being re-weighed. The segments included crop, proventriculus, gizzard, duodenum, jejunum, ileum, and ceca. Only visually healthy birds were sampled.

2.5 Statistical analyses

The production data were analyzed using SAS® 9.4 (Cary, NC) in a 4 (lighting program) x 2 (gender) factorial arrangement. The experimental unit for analyses of gender was pen, and room for lighting program. Lighting treatment was nested within room. Two replications of each lighting treatment were achieved. Data was tested for normality. An analysis of variance using the PROC MIXED procedure was used to examine the differences between lighting programs, genders, ages and to examine the interactions. Tukey's range test was used to separate means when the ANOVA found significant differences between main effects. In addition, the relationships between duration of dark exposure and the dependent variables were tested using PROC REG (Regression) and PROC RSREG (Response Surface Regression). Differences were considered significant when $P \leq 0.05$.

The GIT segment and content percentage data were (log+1) transformed to achieve a normal distribution and statistical differences were based on the log-transformed values. The GIT segment and content weight data, measured only on male broilers, were analyzed as a 4 (lighting program) x 12 (time of day) factorial arrangement with lighting program nested within room. The replicate unit was room. The segments of the small intestine (duodenum, jejunum, and ileum) were analyzed separately as well as combined.

2.6 Results

2.6.1. Production

2.6.1.1. Dark exposure

Growth rate. A significant interaction between dark exposure and gender was noted for body weight at 21d (Table 2.2). Female body weights increased with decreasing darkness, while male body weights peaked with 4D and then decreased with 1D. Table 2.2 lists the growth parameters that showed a significant linear or quadratic relationship with darkness exposure. While no linear or quadratic relationships were noted between dark exposure and growth rate, the analysis of variance effects of dark exposure on body weight are shown in Table 2.4. Chick weights were similar across lighting treatments (43-44g) and genders (43g). Lighting treatments did not result in a difference in body weight at 7 d. At 21 d, birds raised on 10D were lighter than birds from all other treatments. At 31d, the broilers raised on 7D were significantly heavier than those raised on 10D or 1D. The birds raised on 4D were significantly heavier than those raised on 10D, but were not different from birds raised on 7D and 1D.

Feed consumption. A quadratic relationship existed between dark exposure and average feed consumption from 0-31 d (Table 2.3), with a maximum feed intake observed under 4D.

The ANOVA effects of dark exposure on feed consumption are shown in Table 2.5. Analysis of variance revealed that from 7-21 d, 7-31 d, and 0-31 d birds raised on 10D had the lowest feed consumption, but from 21-31 d there was no difference between 10D, 4D or 1D. No difference in feed consumption was found between the birds reared under 1D, 4D or 7D from 21-31 d, 7-31 d or 0-31 d.

Feed efficiency. Dark exposure improved gain to feed corrected for mortality ($G{:}F^{m}$) in a linear fashion from 7-21 d, 21-31 d, 0-31 d and 7-31 d (Table 2.5). Dark exposure improved gain to feed without the mortality correction (G:F) in a linear fashion from 7-21 d (Table 2.7). During the 21-31 d, 0-31 d and 7-31 d periods G:F was effected quadratically by dark exposure (Table 2.7). Birds raised on the longest dark period (10D) were the most feed efficient ($G{:}F^{m}$ and (G:F) for both the 0-31 d and 7-31 d periods. There was no difference between birds raised on 7D, 4D or 1D (Table 2.6 and 2.7, respectively).

Mortality. A quadratic relationship existed for the 21-31 d, 0-31 d, and 7-31 d periods, with the highest mortalities occurring under the 4D and 7D lighting treatments (Table 2.3). Analysis of variance did not reveal a significant effect of dark exposure (Table 2.8). The causes of mortality were not determined for this experiment.

2.6.1.2. Gender

The differences between genders were as expected and will only be briefly discussed. Males had heavier body weights from 7 d onward (Table 2.4) and consumed more feed (Table 2.5) at each of the measured periods compared to females. Males were more feed efficient when corrected for mortality at each measured period, except from 21-31d, compared to females (Tables 2.6). Without the mortality correction, males were more feed efficient from 0-7 d and 7-21 d, with no differences observed between the genders for the other time periods (Table 2.7). There was a gender effect observed for mortality for the 21-31d, 0-31d and 7-31 d periods with males having higher mortality (Table 2.8).

2.6.2. Organ and content weight

2.6.2.1. Dark exposure

The interactions between dark exposure and time of day on GIT tissue and digesta content weights are shown in Table 2.9. An interaction between time of day and dark exposure was observed on full crop weight, with the 4D, 7D and 10D treatments reacting in a similar fashion. Birds reared in these treatments demonstrated peaks occurring shortly before the beginning of the scotoperiod. This peak was absent under the 1D treatment. Looking at crop content relative to body weight there was an interaction effect between time of day and lighting treatment. Again, birds on the 4D, 7D and 10D treatments showed a peak in crop content prior to the dark period and an unexplained peak occurred in the 1D birds at a different time (18:00). Full jejunum, full small intestine and empty jejunum weights as well as ileum and small intestine content relative to body weight each showed an interaction effect between time of day and duration of dark exposure with differences in the magnitudes of the reactions resulting in the significant interactions.

Body weights of males used for the GIT component of the experiment (27-28 d) were heaviest in birds reared under 4D (1.89kg) and 7D (1.91kg) compared to 1D (1.87kg) and 10D (1.76kg).

The relative empty crop weight (empty crop weight relative to body weight) changed in a quadratic fashion with duration of dark exposure (Table 2.10). The full crop weight also showed a quadratic response to dark exposure (Table 2.11). The full crop weight (Table 2.11) and empty crop relative to body weight (Table 2.12a) reacted similarly in response to dark exposure, with the heaviest weights found in birds reared under 10D. The crop content increased in a linear fashion with increasing darkness (Table 2.10, Table 2.13a).

A decreasing linear response was observed between increasing duration of dark exposure and the empty proventriculus relative to body weight (Table 2.12a). With regards to the analysis of variance, full proventriculus weight was only different between birds reared under 7D and 10D (Table 2.11). The content of the proventriculus responded in a quadratic fashion to duration of darkness, with higher content for the 7D birds compared to 10D. No differences were found in proventriculus content between the 7D and 10D birds as compared to the birds reared in the 4D and 1D programs (Table 2.13a).

Empty gizzard relative to body weight showed a linear increase as dark exposure increased (Table 2.10) and the same response is shown by the analysis of variance (Table 2.12a). Full gizzard weight increased in a linear fashion with increasing darkness, however the analysis of variance did not show an effect (Table 2.11). The gizzard content shows a linear response with birds raised under 10D having heavier gizzard contents than those raised under 1D (Table 2.13a).

The empty duodenum relative to body weight showed a quadratic response to duration of dark exposure (Table 2.10). The full duodenum weight showed a linear effect, with the smallest weights in birds reared under 10D (Table 2.11). The empty duodenum relative to body weight of birds given 7D was not different from birds from any other treatment, but the 1D treatment resulted in heavier weights than 4D or 10D (Table 2.12a). No effect of dark exposure was observed on duodenum content (Table 2.13a).

Empty jejunum relative to body weight responded quadratically to dark exposure (Table 2.10), with heavier weights in birds reared under the 1D program compared to 4D. Full jejunum weights showed a linear response and were similar in birds reared under 1D, 4D and 7D, but were lower under 10D (Table 2.11). In terms of empty jejunum weight relative to body weight neither the 1D nor 4D treatments differed from 10D or 7D (Table 2.12a). Jejunum content relative to body weight had a linear response to duration of dark exposure (Table 2.10), with the 10D treatment resulting in a lower content than 1D (Table 2.13a).

Full ileum weights responded in a decreasing linear fashion with increasing darkness. However, the analysis of variance found no differences between birds raised on 1D, 4D and 7D and the lowest weights under 10D (Table 2.11). The empty weight of the ileum was unaffected by duration of dark exposure (Table 2.12a). The content relative to body weight of the jejunum and ileum showed decreasing linear responses to increasing dark exposure (Table 2.10).

Full and empty weights of the combined small intestine (duodenum + jejunum + ileum) showed a linear decrease with increasing darkness (Table 2.10, 2.14). The small intestine content relative to body weight also showed a linear decrease with increasing darkness (Table 2.10, 2.14). The empty small intestine relative to body weight decreased as darkness increased (Table 2.14).

The full, empty, and relative weights of the ceca, as well as the content weights, were unaffected by dark exposure (Table 2.11, Table 2.12a, 2.13a).

2.6.2.2. Time of day

Crop content weights relative to body weight (Table 2.13b) were effected by time of day, with a large peak occurring at the end of the photoperiod for birds from all treatments except 1D. During the dark periods the crop contents are lowest just before the beginning of the photoperiod (Figure 2.1). The contents of the gizzard were highest approximately two h after the peak in crop content for the birds reared on 7D and 10D, but occurred around the same time as the crop peak in the 4D treatment (Figure 2.2). Interestingly, the peak in the duodenum content occurs at the same time (2400 h) across all treatments (Figure 2.3). The content of the jejunum is similar for all treatments during the photoperiod. However, during the scotoperiod, there is no effect on the 1D treatment and the remaining treatments show varying degrees of decreasing content before increasing when the lights come on (Figure 2.4). The ileum contents react in a similar fashion to the jejunum and did not completely empty in broilers reared on any of the lighting treatments (Figure 2.5).

2.7 Discussion

For producers, some of the most important aspects of raising broiler chickens are to achieve optimal market body weights and feed efficiency values in conjunction with a low level of mortality. Therefore, production parameters such as body weight, feed consumption, and feed efficiency are considered very important. Management practices that can optimize these parameters are considered valuable and producers are more willing to implement these types of practices.

With regards to production data, the results in this study support those found in earlier studies (Lewis et al., 2008; Schwean-Lardner et al., 2012b). Even at 31 d of age, birds reared under 4D or 7D were heavier that those under 1D or 10D, indicating that compensatory growth had taken place, similar to that found in studies performed by Rozenboim et al. (1999), Sanotra et al. (2002), Classen (2004) and Schwean-Lardner et al. (2012b).

The mechanisms proposed in the previous research for the effects noted when a dark period is used include altering the growth curve to limit early growth, exposure to darkness resulting in physiological changes to the body, including melatonin and other hormone production, increased exercise, and the beneficial effects of sleep itself. Birds raised with a dark period are healthier in terms of reduced skeletal (Sanotra et al., 2002; Schwean-Lardner et al., 2013) and metabolic (Classen, 2004; Brickett et al., 2007; Schwean-Lardner et al., 2013) diseases and improved immune function (Moore and Siopes, 2002; Zheng et al., 2013). These birds are also more active during the photoperiod, spending more time performing exercise and exploratory behaviours, while birds raised on 1D are lethargic and spend most of their time lying down (Schwean-Lardner et al., 2012a). During the scotoperiod, birds raised with longer dark periods are able to sleep/rest without being interrupted, which may improve the quality of their sleep (Schwean-Lardner et al., 2014). Sleep is thought to be important in terms of growth, energy conservation, tissue repair, and brain function (Adam, 1980). At 21 d, the birds in the current study raised on 23L:1D weighed the same as birds on 4D and 7D, however by 31 d the birds raised with 4D and 7D reached higher weights than those with 1D or 10D. Providing a dark period results in a decrease in early growth rate of the birds, with compensatory gain resulting in equal or greater body weights than those achieved through continuous or near-continuous lighting programs (Classen, 2004; Downs et al., 2006; Schwean-Lardner et al., 2012b).

In addition to the current knowledge on how darkness exposure affects production performance, it is also possible that changes in feeding behaviour could be partially responsible for altering productivity. When birds are offered a dark period greater than or equal to 4 h they learn to anticipate these dark periods (Classen et al., 2016) and will fill their crops to maintain a source of feed throughout the dark period (Buyse et al., 1993). The birds also increase their feeding activity during the photoperiod (Schwean-Lardner et al. 2012a). This adaption in feeding behaviour in response to an extended dark period could partially account for the increased body weight of the birds given 4D and 7D of darkness. As for the birds reared under 23L:1D, who have visual access to the feeders for 23 h per day, it is possible that their body weights were not as high as birds offered moderate dark periods because they lacked the benefits that dark periods provide.

The feed consumption data collected in this study showed that while birds reared under 1D consumed more feed than birds reared under 10D, there was no difference between the broilers reared under the 1D, 4D, or 7D treatments. This suggests that birds reared on these moderate dark periods can adjust their feed intake to account for the shorter daylength by anticipating the scotoperiod and increasing their feeding activity during the photoperiod (Schwean-Lardner et al., 2012a). Birds raised on 23L:1D are less mobile than birds raised with longer dark periods, possibly due to sleep deprivation or poorer skeletal health (Schwean-Lardner et al., 2012a, 2013), and this may partially account for the lack of difference in feed intake between the 1D treatment and the 4D and 7D treatments. Schwean-Lardner et al. (2012a) used the same lighting programs and grew birds to a similar age and found that birds raised on 4D consumed the most feed followed by 1D, then 7D, with 10D birds eating the least in the first experiment. However, during a second experiment the authors obtained the same results as the current study.

Feed efficiency was highest in birds reared under the longest dark period, which is also supported by previous studies (Classen, 2004; Lewis et al., 2008; Schwean-Lardner et al., 2012b). It has been suggested that the improved feed efficiency observed under longer dark periods is partially due to a reduction in maintenance requirements as a result of a more concave growth curve (Buyse et al., 1996). The improved efficiency has also been related to reduced metabolic rates and activity during the dark period (MacLeod et al., 1980; Classen, 2004) and less carcass fat (Classen, 2004). It is also possible that melatonin could play a role in improving feed efficiency (Schwean-Lardner et al., 2014). Endogenous melatonin peaks during the dark period and may induce the onset of sleep (Bermudez et al., 1983), which could both reduce metabolic rate and improve health. Apeldoorn et al. (1999) added exogenous melatonin to a broiler diet and observed improved feed efficiency, possibly caused by a decrease in energy expenditure. The same study compared continuous lighting to an intermittent 6 (1L:3D) program and found that improved metabolism and lower energy expenditure related to physical activity of birds reared on the intermittent lighting schedule caused the improvement in feed efficiency.

This work showed a quadratic relationship between dark exposure duration and bird mortality, with the highest mortalities occurring in the 4D and 7D treatments. However, previous work shows that increasing levels of darkness result in a reduction in mortality (Schwean-Lardner et al., 2012b). The discrepancy between the current study and the Schwean-Lardner et al. (2012b) study may have been due to a difference in sample size and number of replicates used. The earlier study was much larger than the current one and therefore, is likely more accurate. The improvement in mortality may be a result of the early shift in the growth curve rather than a decrease in rapid growth as was previously thought, and also darkness itself, independent of growth rate, results in reduced mortality (Schwean-Lardner et al., 2013).

Periods of light and dark can be used to manipulate feed intake (Sacranie et al., 2012) because birds tend to only feed during the photoperiod, with little nocturnal feeding occurring even with dark periods of 10 h (Buyse et al., 1993). Therefore, the dark period acts as a type of feed restriction/deprivation period. However, birds raised on long (>10 h) or continuous dark periods can learn to feed during the dark period (Cherry and Barwick, 1962). Birds raised with a dark period learn to anticipate the scotoperiod and will increase their feed intake prior to lights turning off, assuming the lights turn off at the same time each night. These birds will also show increased feeding activity when the lights turn on again (May and Lott, 1992). Schwean-Lardner et al. (2014) did not see any anticipatory feeding activity in birds raised under 1D which was in agreement with this work, however whereas they also did not see any anticipatory feeding in birds raised on 4D, the current work did show this pattern of feeding before lights were turned off. One reason for this discrepancy could be the difference in techniques used with the current results obtained from GIT content weights of individual birds versus scan sampling of feeding behaviour. Birds may not have increased the frequency of feeding before dark, but instead increased the amount of feed consumed during each bout.

With minimal scotoperiod feeding typically noted in broilers, birds need to maintain a source of energy for the majority of the dark period. They are able to do so by retaining feed primarily in their crops with some ingesta also present in the proventriculus and gizzard (Buyse et al., 1993). If birds are fed an ad libitum diet and reared on constant or near-constant light, the majority of feed may bypass the crop (Savory, 1985; Chaplin et al., 1992; Classen et al., 2016) and possibly the proventriculus and gizzard if empty, and enter the small intestine almost directly (Jackson and Duke, 1995). It is interesting to note that the crop contents of birds under 10D remained highest throughout the light period, likely in an attempt to compensate for the reduced hours of light. It also appears that these birds reach their peak crop content very close to the beginning of the dark period, whereas content for birds reared on 4D and 7D peaked a few hours before the dark period. Again, this could possibly indicate that the longer dark period challenged birds to consume enough feed during the light period. However, this did not result in birds eating a substantial amount during the dark period. The gizzard content data shows that on longer dark periods (7D and 10D) the peak occurs a few hours after the peak in crop content, whereas on 4D the peaks appear to occur very close to each other. It is possible that 4D is not long enough to require birds to retain feed in their crop for any extended period. The duodenum content data appears to provide evidence of the highly regulated control of feed from the gizzard, with little variation between treatments.

Using a lighting program with an adequate dark period (>4 h) results in storage of feed in the crop, a longer retention time, lower pH, and increased ingesta moisture content (Svihus, 2014; Classen et al., 2016), which can affect nutrient digestibility. Lower pH values, due to organic acids produced by Lactobacilli species, may result in improved nutrient absorption and feed utilization of the feed stored in the crop (Svihus et al., 2013). Maintaining the crop's stable and dominant Lactobascilli population is beneficial in preventing colonization of harmful bacteria including Salmonella and other Enterobacteriaceae (Hinton et al., 2000a,b) and therefore may have a beneficial effect on GIT health (Classen et al., 2016). The use of darkness in a lighting program impacts how birds prepare for that period and this could be one mechanism by which GIT health is improved. Periods of time where birds are not consuming food can result in a shift in the microbial populations in the crop from beneficial to detrimental bacterial species (Classen et al., 2016) and a long dark period can act as one of these periods. However, birds raised with 11D, compared to 6D and 1D, had lower crop pH and showed an increase in the abundance of Lactobacillus species (Dalal et al., 2016). It is possible that the anticipatory feeding pattern shown by birds raised with long dark periods allows birds to store feed in their crops for the majority of the scotoperiod and thus avoid colonization by harmful bacterial species. Barash et al. (1993) fed birds either ad libitum or 1 or 2 times a day and found that meal fed birds had heavier crops and gizzards both with an increased holding capacity. Although birds in this study were fed ad libitum, the dark period represented a period of feed withdrawal, with only negligible feeding occurring during the longer scotoperiods (Shynkaruk, Chapter 3). The empty weights of the GIT segments that were collected reflect changes in musculature as well as development. The current work showed an increase in empty crop and gizzard weights with longer dark periods, likely because of the increased utilization of these sections of the GIT.

The time that feed spends in the crop is very important. The duration of dark exposure affects both the amount of feed that enters the crop as well as the duration of time it remains there (Classen et al., 2016). Feed passage rate is dependent on feeding behaviour, particularly the interval between meals (Svihus, 2015). The average retention time in the crop of broilers fed ad libitum and raised with continuous light is approximately 7 minutes (Shires et al., 1987). Cutler et al. (2005) showed that turkeys raised on 14L:10D had ingesta present in their crops for up to 9 h after the end of the photoperiod. Buyse et al. (1993) also used 14L:10D with broilers and found only small amounts of feed in the crop and proventriculus/gizzard during the photoperiod, however during the scotoperiod this increased to 10.5 and 2.76 times that amount, respectively, with decreasing amounts as the dark period progressed. Hetland and Svihus (2001) showed the average retention time in the digestive tract, excluding the ceca, was 4 to 8 h in broilers raised on continuous light. The results of the present study indicate that feed is still present in the crop, proventriculus and gizzard even after 10 h of dark exposure, with negligible nocturnal feeding occurring. This suggests that birds raised on dark periods up to 10 h in duration are able to maintain a source of energy throughout the dark period. After the 4, 7, and 10 h dark periods the intestinal contents were low and it is advantageous for birds to limit the amount of time that the intestines are near empty. One way this can be achieved is through an increase in feed transit time. The increase in contents in the ileum at the beginning of the photoperiod after 4, 7 and 10 h of darkness illustrate this.

In the present study, the birds reared on longer dark periods had heavier gizzards and because storage is limited in the gizzard, this suggests that the increase in weight may be due to more grinding activity and involvement in regulating feed passage to the rest of the GIT. This is logical because with more crop storage occurring, prior to dark periods, the gizzard will have a larger role to play in regulating the passage of feed to the remainder of the digestive tract and in a grinding capacity, resulting in greater muscle mass. Buyse et al. (1993) showed that feed transit time was slower during the dark period and therefore if the gizzard increases the work it performs this could suggest an improvement in digestion. Future work looking at how different durations of dark exposure effect feed retention time would be useful in assessing the effect of dark exposure on production, nutrient digestibility, and gut health.

The current study found smaller intestinal weights with longer dark periods. While not substantiated it is possible that increased grinding in the gizzard results in a reduction in the surface area needed for absorption in the small intestines. Certain effects may not be seen when examining the weight of the small intestine and interpreting the differences can be difficult, whereas assessing the changes in intestinal function through histology may be more useful (Svihus, 2014). Future research looking at changes in intestinal morphology of birds raised on different dark periods could be beneficial.

In conclusion, this work shows that duration of dark exposure affects productivity and GIT segment and content weights. Moderate dark periods of 4 and 7 h resulted in the highest body weights and similar feed intakes as compared to birds reared under 1 h of darkness. Feed efficiency was highest in birds raised on 10D. Previous studies, which included more replicates and higher bird numbers, demonstrated that feed conversion was improved using both 7 and 10 h of darkness compared to 1 and 4 h of darkness (Schwean-Lardner et al., 2012b). Mortality was highest in the 4D and 7D treatments, but again previous work using a larger sample size and number of replicates found that increasing darkness results in a decrease in mortality (Schwean-Lardner et al. 2012b). It was hypothesized that dark periods would alter GIT segment and content weights. Longer dark periods were associated with larger crops and gizzards. The larger crops are likely due to birds anticipating the dark periods and utilizing their crops for storage. The gizzard, which controls passage of feed into the intestine, must increase its functioning to maintain a source of feed for the duration of the dark period and therefore also increases in size. The intestinal weights were smaller with increasing darkness, which may suggest an improvement in nutrient absorption. It was also hypothesized that longer dark periods would result in a slower feed passage rate and this was confirmed, as indicated by the content weights of different segments of the GIT. The changes in the size of the GIT segments in combination with a longer retention time during the dark period may promote an improvement in digestibility and/or feed efficiency.

2.8 Acknowledgements

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Table 2.1. Ingredients and nutrient composition for starter and grower diets fed to male and female broilers reared to 31 d

Ingredients: (%)	**Starter**	**Grower**
Soybean meal	300.16	193.70
Corn	258.30	35.30
Wheat	150.00	589.91
Peas/lentils	144.30	0.00
Corn DGS	50.00	0.00
Corn gluten meal	0.00	64.50
Meatmeal	40.00	60.00
Barley	22.70	0.00
Canola oil	18.00	40.20
Methionine	3.39	1.66
Salt	2.11	1.45
Selenium	1.50	1.50
Lysine HCL	1.47	3.18
Limestone	1.35	0.00
Choline chloride	1.20	1.42
Vitamin premix[1]	1.06	1.05
Mineral premix[1]	0.87	0.79
Biotin	0.79	0.76
Sodium bicarbonate	0.73	1.95
Mono calcium phosphate	0.00	0.90
L-Threonine	0.57	0.45
Rumensin	0.50	0.50
Vitamin E	0.01	0.01
Nutrients: (%)	**Starter**	**Grower**
AME (kcal/kg)[2]	3050	3200
Crude protein	24.3	23.0
Calcium	0.90	0.91
Non-phytate phosphorus	0.47	0.44
Sodium	0.16	0.16
Arginine	1.41	1.13
True ILD Lysine	1.20	0.91
Methionine + Cystine	0.89	0.77
Threonine	0.70	0.63
Tryptophan	0.20	0.19

[1] Supplied per kilogram of diet: vitamin A (retinyl acetate + retinyl palmitate), 11000 IU; vitamin D_3, 2200 IU; vitamin E (dl-α-topheryl acetate), 30 IU; menadione, 2.0 mg; thiamine, 1.5 mg; riboflavin, 6.0 mg; niacin, 60 mg; pyridoxine, 4 mg; vitamin B_{12}, 0.02 mg; pantothenic acid, 10.0 mg; folic acid, 0.6 mg; and biotin, 0.15 mg; ethoxyquin, 0.625 mg; calcium carbonate, 500 mg.
[1] Supplied per kilogram of feed: iron, 80 mg; zinc, 80 mg; manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; and selenium, 0.3 mg.
ILD= Ileal digestible.

Table 2.2. Interaction between dark exposure and gender on body weight of male and female broilers at 21 d

	Gender	Dark Exposure (D)			
		1	4	7	10
Body weight,	M	1.13	1.16	1.15	1.09
21 d	F	1.05	1.04	1.04	0.99

D = Hours of dark over 24h.

Table 2.3. Summary of regression analysis of dark exposure effects on growth parameters in male and female broilers reared to 31 d of age

	Regression		Equation	R^2
	Linear	Quadratic		
Feed intake, 0-31d	-----	0.0383	$Y=0.6082+0.2506x-0.0064x^2$	0.1211
$G{:}F^m$, 7-21d	<.0001	-----	$Y=0.8223-0.0034x$	0.4387
$G{:}F^m$, 21-31d	0.0033	-----	$Y=0.6896-0.0030x$	0.1490
$G{:}F^m$, 0-31d	<.0001	-----	$Y=0.7506-0.0028x$	0.2696
$G{:}F^m$, 7-31d	<.0001	-----	$Y=0.7411-0.0029x$	0.2559
G:F, 7-21d	<.0001	-----	$Y=0.8138-0.0032x$	0.3844
G:F, 21-31d	-----	0.0065	$Y=1.0584-0.0456x+0.0011x^2$	0.2588
G:F, 0-31d	-----	0.0030	$Y=0.9846-0.0301x+0.0007x^2$	0.3731
G:F, 7-31d	-----	0.0035	$Y=0.9782-0.0305x+0.0007x^2$	0.3734
Mortality %, 21-31d	-----	0.0400	$Y=-22.3578+2.6696x-0.0711x^2$	0.0799
Mortality %, 0-31d	-----	0.0110	$Y=-40.2180+5.0314x-0.1364x^2$	0.1161
Mortality %, 7-31d	-----	0.0261	$Y=-30.9166+3.7956x-0.1013x^2$	0.0926

Table 2.4. Effect of dark exposure, gender and their interaction on body weight (kg) of male and female broilers at the indicated ages

	Dark Exposure (D)					Gender (G)			D x G	SEM
	1	4	7	10	P value	M	F	P value		
0d	0.04	0.04	0.04	0.04	0.18	0.04	0.04	0.74	0.57	**<.001**
7d	0.18	0.18	0.18	0.18	0.30	**0.180[a]**	**0.175[b]**	**<.01**	0.37	0.0006
21d	**1.09[a]**	**1.10[a]**	**1.09[a]**	**1.04[b]**	**<.01**	**1.13[a]**	**1.03[b]**	**<.01**	**0.02**	0.008
31d	**2.09[bc]**	**2.12[ab]**	**2.14[a]**	**2.08[c]**	**<.01**	**2.23[a]**	**1.98[b]**	**<.01**	0.20	0.018

[a,b,c] Means with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
D x G = Interaction between dark exposure and gender.
Regression analyses were not significant.

Table 2.5. Effect of dark exposure, gender and their interaction on average feed consumption (kg) of male and female broilers at the indicated periods

	Dark Exposure (D)					Gender (G)			D x G	SEM	Regression
	1	4	7	10	P value	M	F	P value			
0-7d	**0.15^{b}**	**0.15^{ab}**	**0.15^{a}**	**0.15^{ab}**	**<.01**	**0.15^{a}**	**0.15^{b}**	**<.01**	0.51	0.001	
7-21d	**1.22^{a}**	**1.23^{a}**	**1.20^{b}**	**1.12^{c}**	**<.01**	**1.25^{a}**	**1.14^{b}**	**<.01**	0.07	0.001	
21-31d	**1.62^{ab}**	**1.64^{ab}**	**1.66^{a}**	**1.60^{b}**	**0.04**	**1.73^{a}**	**1.53^{b}**	**<.01**	0.48	0.017	
0-31d	**3.01^{a}**	**3.07^{a}**	**3.06^{a}**	**2.88^{b}**	**<.01**	**3.19^{a}**	**2.82^{b}**	**<.01**	0.51	0.028	**Quadratic (0.04)**
7-31d	**2.86^{a}**	**2.91^{a}**	**2.90^{a}**	**2.73^{b}**	**<.01**	**3.03^{a}**	**2.67^{b}**	**<.01**	0.50	0.028	

[a,b,c] Means with common letters do not differ significantly ($P \leq 0.05$).

SEM = Standard error of the mean.

Regression analyses considered significant if $P \leq 0.05$.

D = Hours of dark over 24 h.

D x G = Interaction between dark exposure and gender.

Table 2.6. Effect of dark exposure, gender and their interaction on gain:feed ratio with mortality correction (G:F^{m}) of male and female broilers at the indicated periods

	Dark Exposure (D)					Gender (G)			D x G	SEM	Regression
	1	4	7	10	P value	M	F	P value			
0-7d	0.908	0.907	0.899	0.909	0.27	**0.911**^{a}	**0.901**^{b}	**0.02**	0.38	0.0021	
7-21d	**0.749**^{c}	**0.754**^{c}	**0.763**^{b}	**0.780**^{a}	**<.01**	**0.771**^{a}	**0.752**^{b}	**<.01**	0.12	0.0023	**Linear (<.01)**
21-31d	**0.624**^{b}	**0.628**^{ab}	**0.634**^{ab}	**0.652**^{a}	**0.03**	0.641	0.628	0.07	0.79	0.0035	**Linear (<.01)**
0-31d	**0.690**^{b}	**0.693**^{b}	**0.700**^{b}	**0.716**^{a}	**<.01**	**0.707**^{a}	**0.693**^{b}	**<.01**	0.71	0.0024	**Linear (<.01)**
7-31d	**0.678**^{b}	**0.682**^{b}	**0.689**^{b}	**0.705**^{a}	**<.01**	**0.696**^{a}	**0.681**^{b}	**<.01**	0.74	0.0025	**Linear (<.01)**

G:F^{m} = (final period weight + kg of mortality weight – initial period weight) / period feed consumption.

^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).

SEM = Standard error of the mean.

Regression analyses were considered significant if $P \leq 0.05$.

D = Hours of dark over 24 h.

D x G = Interaction between dark exposure and gender.

Table 2.7. Effect of dark exposure, gender and their interaction on gain:feed ratio without mortality correction (G:F) of male and female broilers for the indicated periods

	Dark Exposure (D)					Gender (G)			D x G	SEM	Regression
	1	4	7	10	P value	M	F	P value			
0-7d	0.905	0.901	0.892	0.905	0.14	**0.906^{a}**	**0.896^{b}**	**0.03**	0.50	0.0023	
7-21d	**0.743^{c}**	**0.748^{bc}**	**0.757^{b}**	**0.773^{a}**	**<.01**	**0.764^{a}**	**0.747^{b}**	**<.01**	0.45	0.0024	**Linear (<.01)**
21-31d	**0.612^{b}**	**0.600^{b}**	**0.612^{b}**	**0.643^{a}**	**<.01**	0.612	0.622	0.18	0.84	0.0041	**Quadratic (<.01)**
0-31d	**0.681^{b}**	**0.676^{b}**	**0.685^{b}**	**0.708^{a}**	**<.01**	0.688	0.687	0.90	0.89	0.0026	**Quadratic (<.01)**
7-31d	**0.669^{b}**	**0.664^{b}**	**0.674^{b}**	**0.697^{a}**	**<.01**	0.676	0.676	0.86	0.89	0.0027	**Quadratic (<.01)**

G:F= (final period weight– initial period weight) / period feed consumption.
^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
Regression analyses were considered significant if $P \leq 0.05$.
D = Hours of dark over 24 h.
D x G = Interaction between dark exposure and gender.

Table 2.8. Effect of dark exposure, gender and their interaction on mortality (% of birds placed) of male and female broilers for the indicated periods

	Dark Exposure (D)					Gender (G)			D x G	SEM	Regression
	1	4	7	10	P value	M	F	P value			
0-7d	0.57	1.42	1.70	1.13	0.12	1.27	1.14	0.84	0.85	0.176	
7-21d	1.46	1.84	1.75	1.27	0.59	1.70	1.47	0.28	0.77	0.223	
21-31d	1.56	2.83	2.69	1.23	0.19	**3.39[a]**	**0.76[b]**	**<.01**	0.98	0.311	**Quadratic (0.04)**
0-31d	3.58	6.09	6.13	3.63	0.10	**6.36[a]**	**3.36[b]**	**<.01**	0.89	0.486	**Quadratic (0.01)**
7-31d	3.02	4.67	4.43	2.50	0.12	**5.08[a]**	**2.23[b]**	**<.01**	0.92	0.402	**Quadratic (0.01)**

[a,b,c] Means with common letters do not differ significantly (*P*≤0.05).
SEM = Standard error of the mean.
Regression analyses were considered significant if *P*≤0.05.
D = Hours of dark over 24 h.
D x G = Interaction between dark exposure and gender.

Table 2.9. Interaction between dark exposure and time of day on GIT segment and content weights of male broilers at 27-28 d

	D	Time (h)											
		0200	0400	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400
Absolute													
Full crop	1	11.98	8.22	8.08	17.63	11.78	11.53	10.65	8.47	31.28	10.50	14.00	9.92
	4	23.82	6.97	12.30	9.33	7.32	7.45	5.60	11.93	22.02	12.90	12.90	29.22
	7	12.37	5.38	8.65	15.37	21.63	14.42	14.80	16.18	13.83	21.00	42.65	32.28
	10	5.47	4.88	19.88	26.40	23.35	32.05	24.45	25.82	39.30	61.00	41.20	11.62
Full jejunum	1	45.78	42.62	43.72	46.77	38.85	42.23	38.32	42.57	43.37	42.43	37.53	40.37
	4	35.27	29.70	39.75	38.68	42.32	38.50	42.83	45.62	36.37	41.87	43.27	38.10
	7	39.92	32.70	36.77	45.38	40.23	37.17	44.30	46.02	40.32	41.30	39.33	41.02
	10	26.47	25.85	36.87	38.83	40.22	39.98	37.75	38.12	44.30	38.85	36.40	35.33
Full small intestine	1	100.05	91.95	95.63	97.62	85.30	96.73	87.05	97.30	94.85	91.75	85.90	95.22
	4	85.83	73.72	86.28	83.55	93.00	85.53	94.05	98.93	86.90	94.00	94.55	88.18
	7	83.78	74.35	80.52	99.40	91.50	83.13	98.17	101.07	90.27	91.90	86.55	95.20
	10	65.07	55.80	79.65	82.85	88.95	86.93	84.77	87.82	101.10	88.47	80.78	79.18
Empty jejunum	1	25.77	23.68	24.98	25.60	22.50	24.83	22.93	23.33	24.63	22.92	23.18	23.17
	4	22.60	21.50	22.23	22.33	24.57	21.07	26.12	23.45	22.08	22.02	24.33	21.02
	7	24.17	23.13	22.37	25.60	22.50	21.78	22.33	26.68	24.22	22.68	24.20	23.05
	10	19.83	18.87	20.68	23.05	24.93	22.93	20.93	20.97	24.87	22.00	22.93	19.97
Relative													
Crop content	1	0.38	0.22	0.18	0.69	0.41	0.37	0.29	0.21	1.63	0.26	0.40	0.23
	4	0.93	0.15	0.41	0.30	0.13	0.20	0.05	0.45	0.83	0.40	0.39	1.33
	7	0.37	0.02	0.23	0.52	0.87	0.46	0.45	0.56	0.42	0.73	1.78	1.39
	10	0.26	0.02	0.93	1.22	0.97	1.49	1.01	1.22	1.83	2.92	1.72	0.39

Table 2.9. Interaction between dark exposure and time of day on GIT segment and content weights of male broilers at 27-28 d

	D	Time (h)											
		0200	0400	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400
Relative													
Ileum	1	0.91	0.79	0.80	0.69	0.81	0.99	0.84	1.14	0.86	0.84	0.80	1.09
content	4	0.78	0.54	0.69	0.77	0.96	0.91	0.80	0.96	0.88	0.10	0.83	0.88
	7	0.52	0.42	0.39	1.02	0.95	0.83	0.96	0.99	0.82	0.84	0.60	1.01
	10	0.50	0.21	0.71	0.70	0.99	0.92	0.84	1.03	1.20	0.94	0.70	0.84
Small	1	2.00	1.88	1.84	1.96	1.78	2.05	1.70	2.25	1.88	1.99	1.60	2.14
intestine	4	1.46	1.02	1.65	1.77	1.99	2.02	1.75	2.19	1.69	2.12	1.94	1.93
content	7	1.38	0.98	1.24	2.12	1.99	1.82	2.15	2.04	1.73	1.92	1.39	2.20
	10	0.94	0.70	1.73	1.73	1.88	1.98	1.84	2.12	2.30	1.96	1.46	1.83

Interactions were considered significant when $P \leq 0.05$.

D= Hours of dark over 24 h.

Relative = (full tissue weight – empty tissue weight) / body weight of the bird.

Small intestine= duodenum + jejunum + ileum.

Table 2.10. Summary of regression analysis on GIT segment and content weights of male broilers at 27-28 d

	Regression		Equation	R^2
	Linear	Quadratic		
Absolute				
Full crop	-----	0.0348	$Y=113.8080-9.1381x+0.2064x^2$	0.1166
Full gizzard	0.0264	-----	Y=49.6485+0.4032x	0.0172
Full duodenum	<.0001	-----	Y=10.8540-0.1722x	0.0772
Full jejunum	0.0002	-----	Y=30.1035-0.5127x	0.0480
Full ileum	0.0009	-----	Y=26.7067-0.4336x	0.0377
Full small intestine	<.0001	-----	Y=67.6642-1.1185x	0.0601
Empty small intestine	<.0001	------	Y=45.8838-0.4888x	0.0551
Relative				
Empty crop	-----	0.0002	$Y=1.1968-0.2512x+0.0061x^2$	0.1476
Empty proventriculus	0.0385	-----	Y=-1.0446+0.0075x	0.0149
Empty gizzard	<.0001	-----	Y=0.5287-0.0105x	0.0596
Empty duodenum	-----	0.0444	$Y=0.1743-0.0702x+0.0020x^2$	0.0254
Empty jejunum	-----	0.0409	$Y=0.7255-0.0608x+0.0017x^2$	0.0194
Contents of crop	<.0001	-----	Y=1.5195+0.1492x	0.0888
Contents of proventriculus	-----	0.0279	$Y=-9.2890+0.6662x-0.0169x^2$	0.0311
Contents of gizzard	0.0005	-----	Y=0.3820-0.0365x	0.0413
Contents of jejunum	0.0055	-----	Y=-0.5969-0.0215x	0.0266
Contents of ileum	0.0173	-----	Y=-0.7494-0.0241x	0.0196
Contents of small intestine	0.0192	-----	Y=-4.4661-0.0655x	0.0306

Absolute = absolute tissue weight.
Relative = (full tissue weight – empty tissue weight) / body weight of the bird.
Small intestine= duodenum + jejunum + ileum.

Table 2.11. Effect of dark exposure and the interaction between dark exposure and time of day on absolute full and empty GIT segment weights in male broilers at 27-28 d

	Dark Exposure (D)					D x T	SEM	Regression
	1	4	7	10	P value			
Full wt. (g)								
Crop	**12.8^{c}**	**13.5^{bc}**	**18.2^{b}**	**26.3^{a}**	**<.01**	**<.01**	0.93	**Quadratic (0.03)**
Proventriculus	**11.0^{ab}**	**9.9^{ab}**	**11.8^{a}**	**7.8^{b}**	**0.02**	0.39	0.48	
Gizzard	40.2	41.8	42.8	43.9	0.16	0.71	0.61	**Linear (0.03)**
Duodenum	**14.8^{a}**	**14.0^{a}**	**14.5^{a}**	**12.9^{b}**	**<.01**	0.13	0.12	**Linear (<.01)**
Jejunum	**42.0^{a}**	**39.4^{ab}**	**40.4^{a}**	**36.6^{b}**	**<.01**	**0.03**	0.46	**Linear (<.01)**
Ileum	**36.4^{a}**	**35.4^{a}**	**34.8^{ab}**	**32.3^{b}**	**<.01**	0.07	0.44	**Linear (<.01)**
Ceca	14.4	13.5	14.2	13.5	0.32	0.49	0.22	
Empty wt. (g)								
Crop	**5.1^{bc}**	**4.6^{c}**	**5.7^{ab}**	**5.8^{a}**	**<.01**	0.16	0.09	**Linear (<.01)**
Proventriculus	**8.2^{a}**	**7.7^{ab}**	**7.9^{a}**	**7.0^{b}**	**<.01**	0.40	0.13	**Linear (<.01)**
Gizzard	25.3	26.1	26.9	26.2	0.07	0.85	0.22	
Duodenum	**13.2^{a}**	**12.3^{bc}**	**12.8^{ab}**	**11.7^{c}**	**<.01**	0.07	0.12	**Linear (<.01)**
Jejunum	**24.0^{a}**	**22.8^{ab}**	**23.6^{a}**	**21.8^{b}**	**<.01**	**0.03**	0.19	**Linear (<.01)**
Ileum	**19.9^{a}**	**19.6^{a}**	**19.9^{a}**	**18.1^{b}**	**<.01**	0.20	0.20	**Quadratic (0.05)**
Ceca	7.2	7.1	6.8	6.7	0.13	0.09	0.09	**Linear (0.02)**

^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
T = Time of day.
D x T = Interaction between dark exposure and time of day.

Table 2.12a. Effect of dark exposure on empty GIT segment weight as a percentage of body weight for male broilers at 27-28 d

	Dark Exposure (D)					SEM	Regression
	1	4	7	10	P value		
Crop	**0.27^{bc}**	**0.24^{c}**	**0.30^{b}**	**0.33^{a}**	**<.01**	0.005	**Quadratic (<.01)**
Proventriculus	0.44	0.41	0.41	0.40	0.09	0.006	**Linear (0.04)**
Gizzard	**1.36^{b}**	**1.39^{b}**	**1.42^{ab}**	**1.49^{a}**	**<.01**	0.012	**Linear (<.01)**
Duodenum	**0.71^{a}**	**0.65^{b}**	**0.67^{ab}**	**0.66^{b}**	**<.01**	0.006	**Quadratic (0.04)**
Jejunum	**1.28^{a}**	**1.21^{b}**	**1.24^{ab}**	**1.24^{ab}**	**0.03**	0.009	**Quadratic (0.04)**
Ileum	1.06	1.04	1.05	1.03	0.69	0.010	
Ceca	0.39	0.38	0.36	0.38	0.11	0.005	

^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
No interaction between dark exposure and time of day was observed.

Table 2.12b. Effect of time of day on empty GIT segment weight as a percentage of body weight for male broilers at 27-28 d

	Time of day (h)													SEM
	0200	0400	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400	P value	
Crop	**0.26**^{bc}	**0.25**^{c}	**0.25**^{c}	**0.28**^{bc}	**0.29**^{abc}	**0.30**^{abc}	**0.29**^{abc}	**0.27**^{bc}	**0.30**^{abc}	**0.33**^{ab}	**0.35**^{a}	**0.26**^{bc}	**<.01**	0.005
Prov.	**0.41**^{b}	**0.39**^{b}	**0.40**^{b}	**0.39**^{b}	**0.44**^{ab}	**0.43**^{b}	**0.52**^{a}	**0.40**^{b}	**0.39**^{b}	**0.40**^{b}	**0.43**^{b}	**0.37**^{b}	**<.01**	0.006
Gizzard	**1.40**^{ab}	**1.46**^{ab}	**1.44**^{ab}	**1.37**^{ab}	**1.48**^{ab}	**1.41**^{ab}	**1.53**^{a}	**1.36**^{ab}	**1.33**^{b}	**1.40**^{ab}	**1.43**^{ab}	**1.34**^{b}	**0.02**	0.012
Duo.	**0.71**^{a}	**0.66**^{ab}	**0.68**^{a}	**0.69**^{a}	**0.70**^{a}	**0.67**^{ab}	**0.71**^{a}	**0.70**^{a}	**0.70**^{a}	**0.62**^{ab}	**0.67**^{ab}	**0.59**^{b}	**<.01**	0.006
Jejunum	**1.20**^{bc}	**1.18**^{bc}	**1.21**^{abc}	**1.35**^{a}	**1.31**^{ab}	**1.28**^{abc}	**1.25**^{abc}	**1.27**^{abc}	**1.27**^{abc}	**1.18**^{bc}	**1.23**^{abc}	**1.16**^{c}	**<.01**	0.009
Ileum	**0.98**^{b}	**1.01**^{ab}	**1.09**^{ab}	**1.14**^{a}	**1.05**^{ab}	**1.05**^{ab}	**1.09**^{ab}	**1.07**^{ab}	**1.06**^{ab}	**1.01**^{ab}	**1.01**^{ab}	**0.98**^{b}	**0.04**	0.010
Ceca	**0.39**^{ab}	**0.40**^{ab}	**0.40**^{ab}	**0.39**^{ab}	**0.40**^{ab}	**0.37**^{ab}	**0.43**^{a}	**0.35**^{b}	**0.35**^{ab}	**0.34**^{b}	**0.34**^{b}	**0.36**^{ab}	**0.01**	0.005

^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).
Prov. = Proventriculus.
Duo.= Duodenum.
SEM = Standard error of the mean.
No interaction between dark exposure and time of day was observed.

Table 2.13a. Effect of dark exposure and interaction between dark exposure and time of day on GIT content as a percentage of body weight in male broilers at 27-28 d

	Dark Exposure (D)					D x T	SEM	Regression
	1	4	7	10	P value			
Crop	**0.44[b]**	**0.46[b]**	**0.65[b]**	**1.14[a]**	**<.01**	**<.01**	0.048	**Linear (<.01)**
Proventriculus	**0.14[ab]**	**0.12[ab]**	**0.20[a]**	**0.05[b]**	**0.05**	0.40	0.020	**Quadratic (0.03)**
Gizzard	**0.80[b]**	**0.84[ab]**	**0.84[ab]**	**1.00[a]**	**0.02**	0.51	0.026	**Linear (<.01)**
Duodenum	0.08	0.09	0.09	0.07	0.19	0.08	0.004	
Jejunum	**0.96[a]**	**0.87[ab]**	**0.88[ab]**	**0.84[b]**	**0.05**	0.11	0.018	**Linear (<.01)**
Ileum	0.88	0.83	0.78	0.80	0.12	**<.01**	0.018	**Linear (0.02)**
Ceca	0.39	0.34	0.39	0.39	0.36	0.42	0.010	

[a,b,c] Means with common letters do not differ significantly ($P\leq0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
T = Time of day.
D x T = Interaction between dark exposure and time of day

Table 2.13b. Effect of time of day on GIT content as a percentage of body weight in male broilers at 27-28 d

	Time (h)													SEM
	0200	0400	0600	0800	1000	1200	1400	1600	1800	2000	2200	2400	P val.	
Crop	**0.42**^{cd}	**0.10**^{d}	**0.44**^{cd}	**0.68**^{abc}	**0.60**^{bcd}	**0.63**^{abcd}	**0.45**^{cd}	**0.61**^{abcd}	**1.18**^{a}	**1.08**^{ab}	**1.07**^{ab}	**0.84**^{abc}	**<.01**	0.048
Prov.	0.18	0.08	0.10	0.08	0.14	0.10	0.26	0.08	0.18	0.12	0.16	0.08	0.40	0.020
Gizz.	**0.85**^{abcd}	**0.69**^{cd}	**0.87**^{abcd}	**0.74**^{bcd}	**0.96**^{abc}	**0.87**^{abcd}	**1.02**^{ab}	**0.67**^{d}	**0.86**^{abcd}	**0.81**^{abcd}	**1.06**^{a}	**1.04**^{a}	**0.01**	0.026
Duo.	**0.07**^{b}	**0.06**^{b}	**0.07**^{b}	**0.09**^{a}	**0.06**^{b}	**0.11**^{b}	**0.06**^{b}	**0.07**^{a}	**0.06**^{a}	**0.11**^{b}	**0.07**^{a}	**0.17**^{a}	**<.01**	0.004
Jej.	**0.70**^{bc}	**0.59**^{c}	**0.90**^{ab}	**1.02**^{a}	**0.92**^{ab}	**0.95**^{ab}	**0.95**^{ab}	**1.05**^{a}	**0.90**^{ab}	**0.98**^{a}	**0.80**^{abc}	**0.90**^{ab}	**<.01**	0.018
Ile.	**0.68**^{cde}	**0.49**^{e}	**0.65**^{de}	**0.80**^{abcd}	**0.93**^{ab}	**0.91**^{abc}	**0.86**^{abcd}	**1.03**^{a}	**0.94**^{ab}	**0.91**^{abc}	**0.73**^{bcde}	**0.96**^{ab}	**<.01**	0.018
Ceca	0.37	0.46	0.37	0.35	0.38	0.41	0.37	0.37	0.35	0.32	0.39	0.36	0.64	0.010

^{a,b,c} Means with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
Prov. = Proventriculus.
Gizz. = Gizzard.
Duo. = Duodenum.
Jej.= Jejunum.
Ile. = Ileum.
P val. = P value.

Table 2.14. Effect of dark exposure and interaction between dark exposure and time of day on small intestine segment and content weights in male broilers at 27-28 d

	Dark exposure (D)					D x T	SEM	Regression
	1	4	7	10	P value			
Absolute wt. (g)								
Full	**93.3[a]**	**88.7[a]**	**89.7[a]**	**81.8[b]**	**<.01**	**0.03**	0.90	**Linear (<.01)**
Empty	**57.1[a]**	**54.7[a]**	**56.3[a]**	**51.6[b]**	**<.01**	0.12	0.41	**Linear (<.01)**
Relative wt. (%)								
Empty	**3.05[a]**	**2.93[ab]**	**2.95[ab]**	**2.90[b]**	**0.02**	0.14	0.019	
Content	**1.92[a]**	**1.79[ab]**	**1.75[ab]**	**1.70[b]**	**0.05**	**0.02**	0.033	**Linear (0.02)**

Absolute = absolute tissue weight.
Relative = (full tissue weight – empty tissue weight) / body weight of the bird.
Small intestine= duodenum + jejunum + ileum.

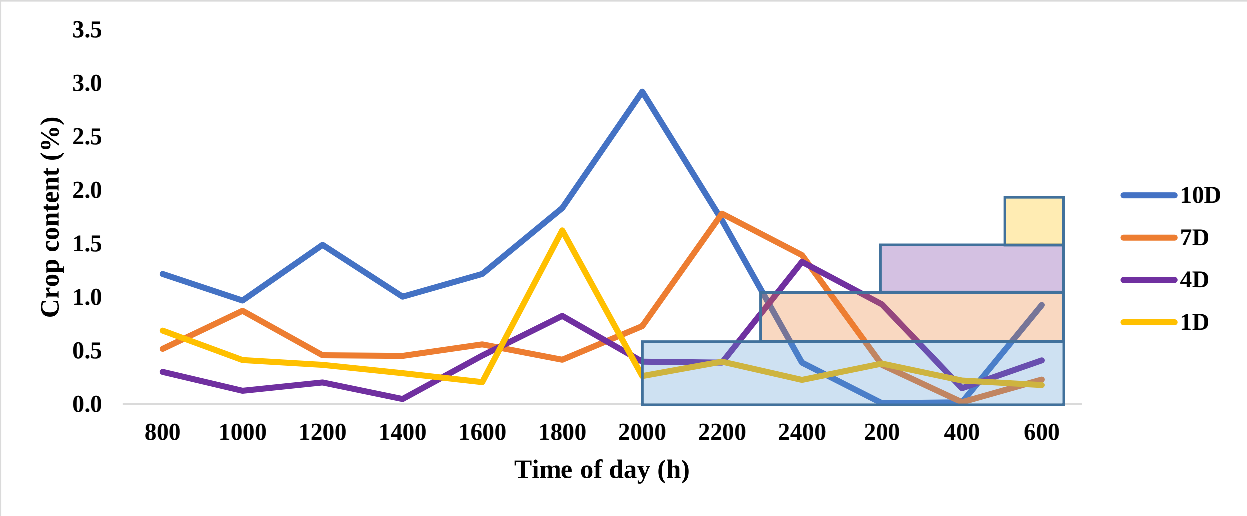

FIGURE 2.1. Effect of dark exposure and time of day on crop content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

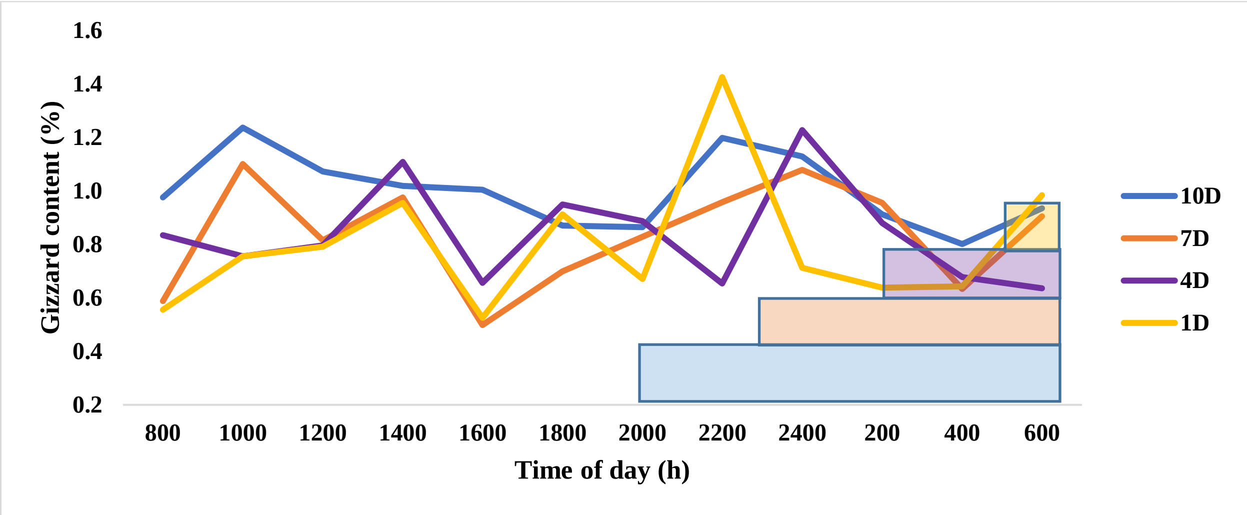

FIGURE 2.2. Effect of dark exposure and time of day on gizzard content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

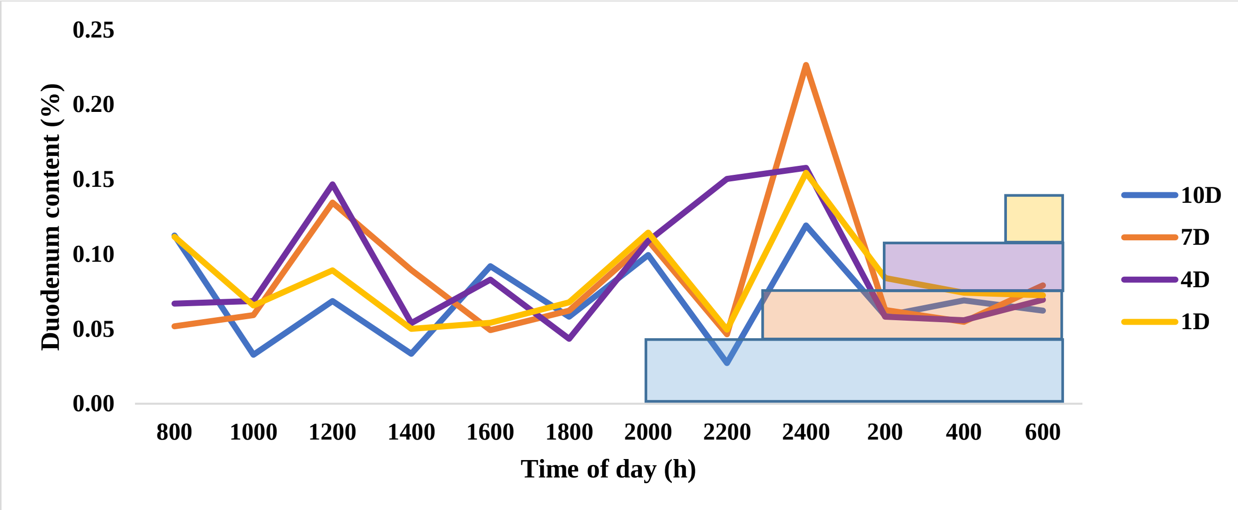

FIGURE 2.3. Effect of dark exposure and time of day on duodenum content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

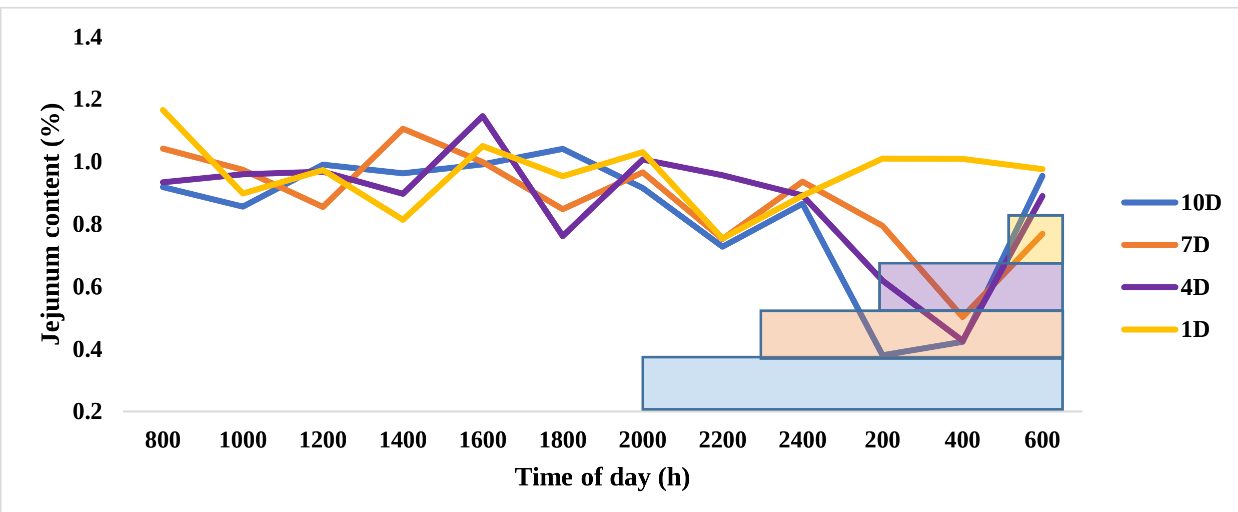

FIGURE 2.4. Effect of dark exposure and time of day on jejunum content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

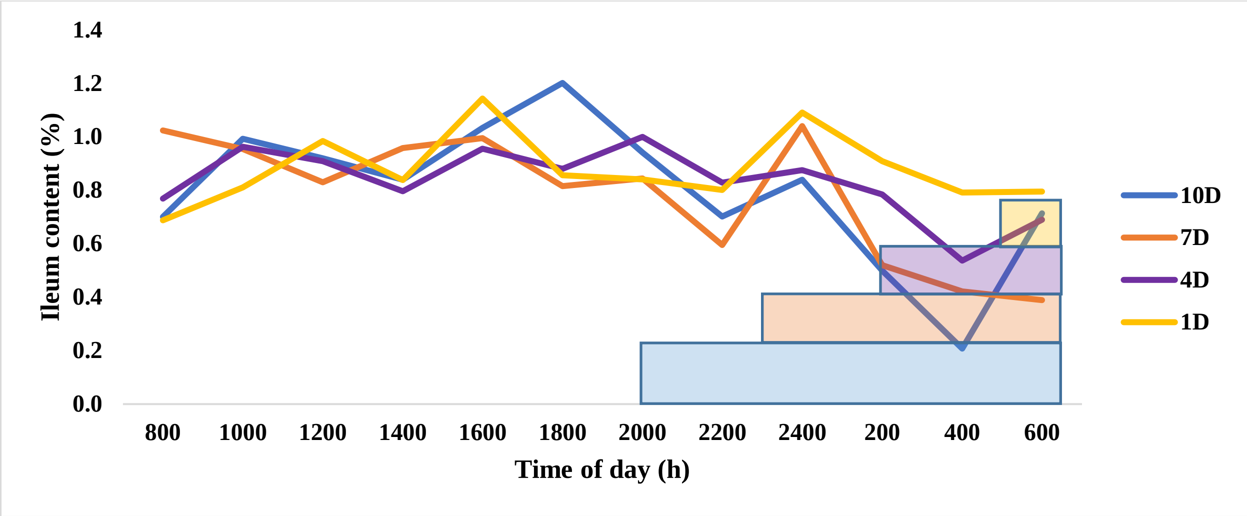

FIGURE 2.5. Effect of dark exposure and time of day on ileum content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

3.0 Chapter 3: Effect of dark exposure on the feeding and drinking behaviour of broiler chickens

Studying the behaviour of an animal helps to identify how they adapt to changes in their environment. Providing broilers with increasing levels of darkness forced them to adapt their feeding behaviours. This adaptation in behaviour can aid in understanding some of the effects identified in Chapter 2, in relation to both productivity and GIT segment and content weights.

3.1 Abstract

The impact of darkness on broiler feeding behaviour was examined using Ross 308 broilers, in a 4x2x2 factorial arrangement of dark exposure (23h light:1h dark (1D), 4D, 7D and 10D), age (2, 4 wk), and gender. One male (n=59) and one female (n=66) pen were observed in each of 8 identical rooms (2 replications per lighting program). Behaviour was recorded using infrared video cameras, which captured an entire pen and recorded in continuous real-time mode for 24 h. At each age, individually marked birds (n=5 per pen) were observed via the video recordings, using focal scanning, with number, duration and frequency of feeding bouts quantified. The effect of age, gender, dark exposure and their interactions were analyzed using Proc Mixed of SAS 9.4, with Tukey's range test used to separate means. Dark exposure data were also analyzed using regression analyses. Differences were considered significant when $P \leq 0.05$. The number of feeding bouts per hour of the photoperiod increased with increasing dark exposure. The number of bouts per hour decreased with age during the photoperiod for all treatments. Males exhibited more feeding bouts per hour than females across all treatments and at both ages. Feeding during the scotoperiod was only observed at 4 wk on the 7D and 10D treatments and was negligible. Feed bout length was unaffected by dark exposure or gender, however older birds had longer bouts. Male birds had shorter feed bout intervals than females. The interval between feeding bouts increased with age. The total time spent at the feeder during the photoperiod was only effected by dark exposure, with birds reared on 1D spending more time at the feeder than birds on 10D. Visually, feeding patterns indicated that birds exposed to dark periods of 7 and 10 h were able to anticipate the scotoperiod and increased their feeding activity prior to dark, which is supported by digesta content weights. Birds reared on 4 h of dark appeared to show an intermediate anticipatory response, whereas birds reared on 1 h of dark did not increase their feeding frequency prior to dark. In conclusion, both duration of dark exposure, age, and gender impact broiler feeding behaviour. Shorter dark periods result in longer feeding bout intervals and fewer feeding bouts during the photoperiod. As birds age, the number of visits to the feeder are reduced with longer intervals between visits and longer feed bout lengths. Males feed more often than females.

Key words: dark exposure, feeding behaviour, broilers

3.2 Introduction

Studying changes in behaviour is a common and effective method for assessing the welfare of an animal. Many management practices used in the poultry industry can affect behavioural expression and therefore bird welfare. For example, providing broilers with a dark period as opposed to continuous or near-continuous light, results in a change in their behaviour (Schwean-Lardner et al., 2012a). Birds reared on longer dark periods spend more time performing exercise, exploratory and comfort behaviours (Schwean-Lardner et al., 2012a), which can have positive influences on the bird's well-being. Light is one of the most important external factors that stimulates and regulates biological and behavioural rhythms in poultry (Sanotra et al., 2002; Olanrewaju et al., 2006; Schwean-Lardner et al., 2012, 2014). Providing birds an adequate and regular lighting program allows them to maintain a diurnal rhythm and organize patterns of behaviour, such as feeding (Sanotra et al., 2002).

Studies have shown that birds raised with a dark period are more active during the photoperiod (Schwean-Lardner et al., 2012a) and are able to sleep/rest uninterrupted during the scotoperiod (Malleau et al., 2007). This can contribute to a number of health benefits, which also improves bird welfare. For example, providing darkness results in improved skeletal development (Brickett et al., 2007; Schwean-Lardner et al., 2013), ocular health (Lewis and Gous, 2009; Schwean-Lardner et al., 2013; Leis e al., 2016) and a reduction in metabolic disease (Classen, 2004; Schwean-Lardner et al., 2013), skeletal disease (Sanotra et al., 2002), and overall mortality (Classen, 2004; Schwean-Lardner et al., 2013).

Broilers raised with dark periods of adequate length (>4 h) are able to anticipate scotoperiods and will increase their feeding activity prior to darkness (Duve et al.; 2011; Schwean-Lardner et al., 2014) in order to retain feed in their gastrointestinal tract for the majority of the dark period (Duve et al., 2011). An increase in feeding activity also occurs after the scotoperiod ends. The majority of feeding behaviour occurs during the photoperiod, with scotoperiod feeding typically occurring only during longer dark periods (Savory, 1976; Lewis et al., 2009a; Deep et al., 2012; Schwean-Lardner et al., 2012a). The presence of scotoperiod feeding could indicate that birds are experiencing a state of hunger and/or that they have met their sleep/rest requirement (Schwean-Lardner et al., 2012a).

Previous work has shown that providing darkness affects broiler feeding behaviour (May and Lott, 1992; Buyse et al., 1993; Schwean-Lardner et al., 2012a, 2014), but the specifics of how this occurs, including feed bout length, interval, duration and pattern over a 24 h period are still unknown to the author's knowledge. The current study used focal sampling to continuously monitor the feeding behaviour of individual birds over 24 h at 2 and 4 wk of age, whereas many previous studies used scan sampling at various intervals. The objective of this research was to study the effect of varying levels of darkness on feeding behaviour parameters at different ages in male and female broiler chickens.

3.3 Materials and Methods

3.3.1. Experiment

The experimental protocol for this trial was approved by the University of Saskatchewan Animal Care Committee and was performed under the recommendations of the Canadian Council of Animal Care (1993) as specified in the Guide to the Care and Use of Experimental Animals.

3.3.2. Housing and management

On d 0, 1,888 male and 2,112 female Ross x Ross 308 broilers were randomly distributed among eight rooms (12.19m x 7.01m) upon arrival at the University of Saskatchewan Poultry Centre, and were reared there until 31 d of age. Each room was separated into 8 pens (2.3m x 2.0 m) with 4 pens assigned to males and 4 to females. Pens had an estimated final stocking density of 32kg/m^2 (66 females per pen; 59 males per pen) based on 32 d weights listed under the Ross Performance Objectives (Aviagen, 2014). Straw was used as the litter source. From placement to d 7, all birds were maintained on 1 h of darkness and on d 7, the lighting treatments were initiated. The lighting treatments used were 23L:1D (1D), 20L:4D (4D), 17L:7D (7D) and 14L:10D (10D), with darkness provided in one continuous period. Light intensity was similar in each room (25 lux to d 7, then 5 lux for the remainder of the trial), with light being provided by incandescent bulbs. Light intensity was 0 lux during the dark period. A 15-minute dawn to dusk system was used in each room. Temperature was set at 33°C on d 0 and was reduced to 21°C by 31 d. Feed was provided ad libitum in tube feeders (circumference of 112 cm), and water via Lubing nipple drinkers (Lubing Systems LP, Cleveland, TN, USA; six nipples per pen) for the duration of the trial. Birds were fed 0.65 kg of a commercial starter ration per bird and then the balance of feed until the end of the trial was a commercial grower ration (Table 2.1).

3.3.3. Data collection

Video recordings were taken using a ceiling mounted infrared video camera system (Panasonic WV-CF224FX; Panasonic Corporation of North America, One Panasonic Way 7D-4, Secaucus, NJ, USA). Genetec Omnicast Software (Genetec Inc., Montreal, Quebec, Canada) was used to play back video data for analyses. The cameras recorded, in continuous real-time mode directly to a computer system for a 24 h period, and captured the entire area of the pen. Each pen that was used for behavioural observation (one male and one female pen per room) had its own camera, however due to a limited number of cables that fed back to the computer, video recordings were taken on consecutive days (female pens on d 1 and male pens on d 2). With two rooms per lighting treatment, this resulted in observation of both the gender and dark exposure replicates. Behaviour was recorded during wk 2 (d 13 and 14) and wk 4 (d 29 and 30).

One male and one female pen per room each contained 5 individually marked birds, which were monitored for behaviour analyses. This resulted in the observations of 10 males and 10 females for each lighting program at each age. The method of sampling used in this experiment was focal sampling, in which each focal individual was chosen randomly, marked and observed for an entire 24 h period and a record of behaviour was made for each individual for the entire period (Altmann, 1974; Martin and Bateson, 1993). Birds were re-marked (new birds were selected if the previously used birds could not be identified or had died) before each observation period to enhance the marks and make analyses of the video easier.

The behaviour of interest in this experiment was feeding behaviour, including number, duration and frequency of feeding bouts. Feeding behaviour and drinking behaviour are often associated, therefore drinking behaviour was also observed. The definition used for feeding behaviour was as follows: Initiation of feeding occurred when a bird's head was located over the rim of the feeder with the head orientated downward. Cessation occurred when a bird's head was located or lifted outside of the feeder. If a second feeding event occurred within 10 s it was considered part of the same feeding bout. If a second feeding event occurred more than 10 s after another feeding event, the two were considered separate bouts. A bout interval of 10 s was chosen based on work conducted by Bokkers and Koene (2003). Initiation of drinking was defined as occurring when a bird's head was located underneath the drinker and orientated upward. A drinking bout ceased when the bird's head was removed from under the drinker. The same bout interval that was used for feeding was also used for drinking.

3.3.4. Statistical analyses

The data was analyzed as a 4 (lighting program) x 2 (gender) x 2 (age) factorial arrangement, with lighting program nested within room. The experimental unit for analyses was pen for gender (1 male and female pen/room/age) and age (2 pens/room/age), and room (2 rooms per treatment) for lighting program. The data were analyzed with an analysis of variance using the PROC MIXED procedure of SAS (SAS® 9.4., Cary, NC) to identify differences between gender and age and to determine the presence of interactions between the variables. Tukey's range test was used to separate means when the ANOVA found significant differences between main effects. In addition, the relationships between the duration of dark exposure and the dependent variables were tested using PROC REG (Regression) and PROC RSREG (Response Surface Regression). All data were tested for normality prior to other analyses and (log+1) transformation was used when necessary. Differences were considered significant when $P \leq 0.05$.

3.4 Results

3.4.1. Feeding Behaviour Parameters

3.4.1.1. Dark exposure

Photoperiod

The interactions between dark exposure, gender, and age are shown in Table 3.1. Significant interactions between duration of dark exposure, gender and age were observed for the average number of feed bouts per hour of the photoperiod. Differences were in the degree of response only. Regression analyses showed that as duration of dark exposure increased the number of visits to the feeder per hour also increased in a linear fashion (Table 3.2), with birds raised on 10D having the highest number of bouts per hour of the photoperiod (Table 3.3). There was no effect of duration of dark exposure on feed bout length (Table 3.4). Regression analyses showed that as duration of darkness decreased, feed bout intervals increased in a linear fashion (Table 3.2 and Table 3.5). The total time spent at the feeder increased in linearly with decreasing levels of darkness (Table 3.2 and Table 3.6). Regression analyses of feeding frequency patterns often showed a quadratic response (Figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, and 3.10). If anticipatory feeding is occurring birds will increase their feeding prior to the scotoperiod. Visual assessment indicated that at 2 wk both male and female birds raised with continuous dark periods of 7D and 10D increased the frequency of their feeding prior to the scotoperiod (Figures 3.1, 3.2, 3.6, and 3.7). At 4 wk, visual assessment only indicated this increase in feeding frequency in females raised under 10D (Figure 3.9). However, the GIT content data (Shynkaruk, Chapter 2) demonstrated that males and females at 4 wk, reared under 7D and 10D, increased the amount of content in their crops before the dark period. However, the response was more pronounced at 2 wk of age and in the female birds. Under the 4D treatment this increase in feeding was not observed visually, however GIT content data (Shynkaruk, Chapter 2) shows that these birds do anticipate the dark period and increase crop contents prior to darkness (Figures 3.5 and 3.8). The feeding frequency data showed a quadratic response for 4 wk old females reared on 1D, however it appears that the increases in feeding occurred during the day and not prior to the dark period (Figure 3.11), which is supported by the GIT data (Shynkaruk, Chapter 2).

Scotoperiod

A number of interactions were noted between dark exposure and age (Table 3.1). The average number of feed bouts per hour of scotoperiod showed a significant interaction between dark exposure duration and age, with birds at 2 wk only feeding during the night period while reared with 10D, and at 4 wk feeding during the night while reared on both 10D and 7D. Dark exposure affected the length of feeding bouts during the scotoperiod differently depending on age, with a difference in magnitude between wk 2 and wk 4 causing the interaction. Total time spent at the feeder during the scotoperiod also showed an interaction between dark exposure and age, with birds at 2 wk only feeding during the 10D period and birds at 4 wk feeding during the 10D period at a higher magnitude as well as during the 7D period. No statistical difference was found between the bout length of the birds exposed to 1D, 4D, or 7D, however it is important to remember that of the birds reared on those three treatments, those given 1 and 4 h of darkness did not feed during the scotoperiod (Table 3.4). There was no difference in scotoperiod feed bout interval between any of the treatments (Table 3.5), however it should be noted again that birds on 1D and 4D did not visit the feeder during the scotoperiod. Total time spent at the feeder was longer for 10D birds, however the values are negligible (Table 3.6).

24 h period

Over a 24 h period, the number of feeding bouts per hour decreased in a linear fashion with increasing darkness (Table 3.3).

3.4.1.2. Gender

Male birds visited the feeder more times per hour of the photoperiod and over 24 h (Table 3.3) and had shorter feed bout intervals than females (Table 3.5). There was no effect of gender on any feeding parameters during the scotoperiod. At 4 wk of age, both male and female broilers were observed feeding during the scotoperiod when reared under 10D, but only males fed during the dark period on 7D at this age.

3.4.1.3. Age

During the photoperiod and over 24 h, birds visited the feeder fewer times per hour as they got older (Table 3.3), had longer feed bout lengths (Table 3.4), and longer feed bout intervals (Table 3.5). During the scotoperiod, older birds visited the feeder more often (Table 3.3), had longer feed bout lengths (Table 3.4) and spent more time at the feeder (Table 3.6).

3.4.2. Drinking Behaviour Parameters

3.4.2.1. Dark exposure

Photoperiod

During the photoperiod, an interaction existed between duration of dark exposure and bird age for drink bout interval, with birds at 4 wk having longer intervals on each lighting program than birds at 2 wk (Table 3.1). A linear increase in drinking bouts per hour was found for the photoperiod (Table 3.2), with birds raised with 10D visiting the drinker the most on a per hour basis, while birds raised on 1D had the lowest number of visits (Table 3.3). There was no effect of dark exposure on drinking bout length (Table 3.4). Drink bout interval showed a linear decrease as more darkness was provided (Table 3.2) with birds raised on 10D and 7D having the shortest drink bout intervals and birds on 1D the longest (Table 3.5).

Scotoperiod

There were no observations of birds drinking during the scotoperiod.

24 h period

A linear response to dark exposure was observed for the number of drinking bouts per hour, with more bouts occurring under longer dark periods (Table 3.3).

3.4.2.2. Gender

No effect of gender was observed on broiler drinking behaviour.

3.4.2.3. Age

As birds aged, they visited the drinker less per hour of the photoperiod and over 24 h (Table 3.3), had longer drink bout lengths (Table 3.4) and longer drink bout intervals (Table 3.5). There was no effect on total time spent at the drinker.

3.5 Discussion

Broilers in today's industry have been heavily selected for increased growth rate, resulting in maximum body weights in a relatively short production cycle. The choice of lighting programs used in broiler production varies significantly, and continuous or near-continuous programs may still be used with the belief that market weights of birds reared under these programs are heavier (Savory, 1976; Lewis and Morris, 2006). However, more current research, which examined the relationship between lighting program and production parameters, disputed this theory and found that birds given moderate dark periods (4 or 7 h) were heavier than those given only 1 h of darkness (Schwean-Lardner et al., 2012b). Similar results to this previous study were obtained in the current experiment (Chapter 2). The majority of reported farms in Saskatchewan, Canada provide 4D for the majority of the rearing period (personal communication, Chicken Farmers of Saskatchewan, April 2017).

Providing broilers with a dark period results in an improvement in skeletal development and health, partially due to a slower growth rate early in life (Classen, 2004; Sanotra et al., 2002). The skeletal improvements may be reflected in a bird's activity level, with birds raised with darkness showing increased activity and exercise behaviours such as walking (Schwean-Lardner et al., 2012a). The current study showed that birds raised on longer dark periods visited the feeder more often per hour and had shorter intervals between bouts. This is in agreement with Schwean-Lardner et al. (2012a) who suggested that birds reared under long dark periods would consume more frequent meals, with one mechanism possibly being improved mobility over birds reared under short dark periods. An increase in activity could result in an increase in expression of normal behaviours such as comfort, exploratory and exercise behaviours, which would be beneficial in terms of physical and mental health and indicate improved welfare. In contrast, when birds are exposed to constant or near-constant light they spend more time lying down, resulting in increased contact with the litter as well as less disturbance and drying of the litter, both of which may result in a higher incidence of breast blisters and hock burns (Schwean-Lardner et al., 2012a). However, another explanation for the differences observed between birds raised on 1D and 10D could be that 10D birds had to eat more often to consume enough feed during the reduced photoperiod. It is possible that the 14L:10D lighting program resulted in a light period that challenged birds to be able to consume enough feed to support optimal growth.

If this was the case it remains unclear why no change in feed bout length was observed because it would be expected that birds would spend more time at the feeder. Rather, it was the birds raised with shorter dark periods who spent more time at the feeder. For example, broilers raised on 10D spent significantly less time at the feeder than birds raised on 1D, which may indicate that birds raised on longer dark periods spend more time performing other behaviours and were more active during the light period. However, it is also possible that birds reared on 10D are more mobile and therefore visit the feeder to eat and then leave. In contrast, birds raised on 1D are less mobile and when they visit the feeder they may spend more time there due to an unwillingness to move. It is important to note that, while birds exposed to 1D did spend the most time at the feeder, data from the previous chapter showed that these birds did not consume more feed than birds reared under 4D or 7D. Therefore, this supports the suggestion by Schwean-Lardner et al. (2012a) that these birds spend more time at the feeder due to a reluctance to move, indicated through gait scores, or from lethargy due to sleep deprivation.

The majority of feeding occurred during the photoperiod, with a negligible amount occurring during the scotoperiod for birds on the treatments with a longer duration of dark exposure, especially as birds got older. Sleep is important for poultry (Blokhuis, 1984), in terms of both quality and quantity (Ayala-Guerrero et al., 2003). In this study, most of the feeding occurred during the photoperiod, which supports that birds choose to spend the majority of the dark period sleeping/resting thus resulting in an improved quantity of sleep. This could be because implementation of longer dark periods creates a diurnal rhythm allowing the birds to organize patterns of behaviour, including feeding. Birds can sleep during the photoperiod (Ayala-Guerrero et al., 2003), however the quality of sleep is impaired, possibly due to a reduction in the production of melatonin (Rattenborg et al., 2005) and/or because birds sleeping during the light period only show EEG waves consistent with quiet sleep, whereas during the dark both active and quiet sleep occur (Ookawa and Gotoh, 1964). Also lighting programs with little or no darkness result in birds being disrupted by pen mates when they attempt to sleep or rest (Malleau et al., 2007). Therefore, the quality of sleep is also improved by providing a dark period. Nocturnal feeding was only observed in the 7D and 10D treatments, and usually only occurred at the older age, with only one focal bird feeding during the dark at the younger age. Also, while nocturnal feeding was negligible, its occurrence suggests that a period of 10D may be more darkness than the broilers required because they exhibited a behaviour that is normally performed during the photoperiod (Schwean-Lardner et al., 2012a). Therefore, a period of 10D may be enough that birds have satisfied their sleep requirement or are experiencing hunger.

The data in this work, combined with the digesta content data from the previous chapter, supports the work of Schwean-Lardner et al. (2012a), that 1 h of darkness is not enough for birds to anticipate the dark period. However, it is not clear whether the birds reared on 1 h of darkness were unable to anticipate the dark period, or simply did not need to adjust their feeding for the short dark period. The authors also found that the addition of 4 h of darkness resulted in an intermediate response, which is in agreement with the feeding behaviour observed in this experiment. However, in the present study, the GIT content data demonstrated that birds on 4 h of darkness do anticipate the dark period. We were unable to measure how much birds consumed during each feeding bout, therefore the discrepancy between the feeding behaviour and GIT data of birds reared under 4 h of dark may be due to birds consuming more feed at each bout prior to darkness rather than increasing the frequency of their bouts. The feeding frequency data indicates that birds raised with 7 and 10 hours of darkness often increased their feeding frequency prior to darkness and the GIT data demonstrated they anticipated the scotoperiod and filled their crops prior to darkness. It should also be noted that the implementation of the lighting programs was at d 7 and the first observation period was at d 14, which means that birds are able to quickly adapt to dark periods as demonstrated by the anticipatory behaviour that was observed after one week of providing dark periods of 4 hour or greater. These changes in feeding pattern indicate that implementing longer dark periods results in a diurnal feeding rhythm, with only minimal feeding occurring during the scotoperiod, therefore allowing a high majority of the flock to sleep/rest. This increased feeding that was observed prior to the scotoperiod, in birds reared on 4 or more hours of darkness, is important because it means that more feed is stored in the crop and therefore more feed is available as an energy source during the dark period (Buyse et al., 1993; Shynkaruk, Chapter 2).

Schwean-Lardner (2012a) also hypothesized that exposure to longer durations of darkness would result in shorter meal durations, however no difference in feed bout length was observed in this study. It is not clear why birds altered the frequency of their feeding and the total time they spent at the feeder but not the length of their feeding bouts to compensate for varying lengths of darkness.

Feeding frequency is reduced as birds' age, potentially because of a reduction in mobility, or an increased GIT capacity. However, Schwean-Lardner et al. (2012a) found that, at an older age, birds raised on 1D walked the least compared to birds given 4D, 7D, or 10D and that these 1D birds were not the heaviest. Therefore, there may be another reason that these birds are less active and it may be that they are lethargic due to sleep deprivation (Schwean-Lardner et al., 2012a). During the scotoperiod however, birds fed more often as they aged. As they got older and heavier, their higher feed requirement could have resulted in an increase in feeding during the dark periods, with birds learning to feed during this period. When older birds fed, they remained at the feeder for a longer period of time. This could be explained again by a decrease in activity shown by larger, older birds. Older birds also have longer feed bout intervals between bouts, again suggesting that as birds get heavier they are less motivated to move. Total time spent at the feeder, during the photoperiod, was unaffected by bird age. The decrease in mobility observed in birds raised without extended dark periods could also be due to a decrease in skeletal health, with birds under 1D having poorer gait and footpad lesion scores as well as an increased number of mortalities due to skeletal issues (Schwean-Lardner et al., 2013).

Differences between genders were not surprising. Males fed more frequently than females, possibly because males have a higher feed requirement. Feed bout length was unaffected by gender. There was no significant difference between the total time spent at the feeder, during the photoperiod or scotoperiod, for either gender.

Little is known about the specific pattern demonstrated by broilers during drinking. Birds raised with longer dark periods visited the drinker more frequently, again indicating that these birds are more active than birds exposed to short dark periods. The increase in the number of visits to the drinker per hour for the 10D birds compared to the 1D birds was much greater than the number of visits to the feeder per hour. This may suggest that when offered a shorter photoperiod, birds may have an increased requirement to consume water rather than feed, which is also supported by the observation that birds also returned to the waterer more frequently than they did the feeder. No drinking activity was observed during the dark period for any of the treatments. It could be that birds have trouble locating the nipples on the drinker in the dark, however previous work looking at raising birds in continuous darkness showed that they did learn to drink during in the dark (Whitley et al., 1985). Therefore, it is possible that a dark period of 10 h was not long enough to require birds to learn to drink during this time. However, Warris et al. (2004) suggested that the correlation they found between feeding and drinking was evidence that dry feed intake stimulates drinking, therefore because birds consumed little to no feed during the scotoperiod it is possible that they did not need to drink during this period. No effect of dark exposure was observed for drink bout length or total time spent at the drinker.

Duration of dark exposure, age, and gender alter how birds feed. Broilers exposed to longer dark periods visited the feeder more times per hour, had shorter intervals between bouts and spent less total time at the feeder. It should also be noted that no differences in feed intake were found between birds raised with 1D, 4D or 7D in the current work. This indicates that birds reared on longer dark periods may spend more time performing other behaviours, including sleeping. While the current study found minimal overall feeding occurring during the scotoperiod of both the 7D and 10D treatments, the increase in scotoperiod activity of 10D birds compared to 7D birds indicates that the former dark period may be more than birds require. This work showed that occurrence of birds nutritive behaviours (feeding and drinking) were reduced by short dark periods (23L:1D). In the current study, a period of at least 7 h darkness was required to stimulate the diurnal pattern of feeding and drinking that was observed at the beginning and end of the photoperiods. The increase in feeding bouts observed before the scotoperiod in this chapter as well as the increase in crop fill shown in Chapter 2 both confirm that birds anticipate dark periods (of at least 4 h) to ensure they have a source of energy throughout the dark period. The negligible nocturnal feeding observed in this study suggests that birds were not experiencing hunger, again indicating that they were able to maintain a source of energy during the dark period. While the 1D treatment resulted in reduced behavioural expression and the 10D treatment may be more than birds require, there was little difference between the 4D and 7D treatments.

In conclusion, dark exposure, gender, and age affect feeding behaviour. Males feed more frequently than females and as birds age they feed less frequently and have longer feed bout lengths, suggesting they are less active as they get older. Dark exposure results in significant changes that may affect GIT health and feed efficiency, with longer dark periods increasing the number of feeding bouts prior to darkness. This in turn allows feed to remain in the GIT throughout the dark period. This research provides support to the importance of using dark programs in broiler production systems.

3.6 Acknowledgements

The authors would like to acknowledge the University of Saskatchewan Poultry Staff for their technical support as well as the Poultry Group for their assistance.

Table 3.1. Interaction between dark exposure, gender and age for nutritive behaviours of male and female broilers studied at weeks 2 and 4

	Gender	Dark Exposure (D)			
Photoperiod		**1**	**4**	**7**	**10**
Avg. number of feed bouts/h	M	3.1	3.6	3.5	5.1
	F	3.0	3.1	3.3	3.5
	Age (wk)				
Photoperiod					
Avg. number of feed bouts/h	2	3.2	3.5	4.2	4.6
	4	2.9	3.1	2.6	4.0
Average drink bout interval (s)	2	1022	868	704	571
	4	1655	1142	832	796
Scotoperiod					
Avg. number of feed bouts/h	2	0.0	0.0	0.00	0.03
	4	0.0	0.0	0.07	0.41
Avg. feed bout length (s)	2	0.0	0.0	0	1
	4	0.0	0.0	16	86
Total time at the feeder (s)	2	0.0	0.0	0	1
	4	0.0	0.0	37	422

D = Hours of dark over 24 h.

Table 3.2. Summary of regression analyses for dark exposure and nutritive behaviours of male and female broilers reared to 31 d

	Regression	Equation	R^2
	Linear P value		
Photoperiod			
Avg. number of feed bouts/h	0.0028	Y=5.8776-0.1268x	0.2604
Avg. number of drink bouts/h	<.0001	Y=9.8144-0.3079x	0.6867
Avg. feed interval	0.0135	Y=409.8167+31.5792x	0.1866
Avg. drink interval	<.0001	Y=-410.3083+73.4542x	0.5103
Total time at feeder	0.0021	Y=2241.2742+280.4592x	0.2748
Scotoperiod			
Avg. number of feed bouts/h	0.0069	Y=0.4903-0.0231x	0.2190
Avg. feed bout length	0.0094	Y=99.8917-4.6833x	0.2041
Total time at feeder	0.0139	Y=460.7083-21.7917x	0.1853
24 h period			
Avg. number of feed bouts/h	0.0494	Y=39.7638+1.3064x	0.1227
Avg. number of drink bouts/h	0.0341	Y=100.8050-1.5175x	0.1411

Table 3.3. Effect of dark exposure, age, gender, and their interactions on average number of feeding and drinking bouts per hour of the photoperiod, scotoperiod, and over a 24 hour period of male and female broilers at weeks 2 and 4

	Dark exposure (D)					Age (A, wk)			Gender (G)			D x A	D x G	SEM
	1	4	7	10	P value	2	4	P value	M	F	P value			
Photoperiod														
avg. # feed bouts/h	**3.1^{b}**	**3.3^{b}**	**3.4^{b}**	**4.3^{a}**	**<.01**	**3.9^{a}**	**3.2^{b}**	**<.01**	**3.8^{a}**	**3.3^{b}**	**<.01**	**0.05**	**0.02**	0.15
avg. # drink bouts/h	**2.8^{d}**	**3.6^{c}**	**4.5^{b}**	**5.6^{a}**	**<.01**	**4.6^{a}**	**3.6^{b}**	**<.01**	4.3	3.9	0.07	0.59	0.32	0.22
Scotoperiod														
avg. # feed bouts/h	**0^{b}**	**0^{b}**	**0.04^{ab}**	**0.20^{a}**	**0.01**	**0.006^{b}**	**0.120^{a}**	**0.03**	0.7	0.5	0.57	**0.03**	0.94	0.030
24 h period														
avg. # feed bouts/h	**71^{a}**	**66^{ab}**	**58^{b}**	**60^{ab}**	**0.04**	**70^{a}**	**58^{b}**	**<.01**	**69^{a}**	**59^{b}**	**<.01**	0.11	0.11	2.249
avg. # drink bouts/h	**64^{b}**	**72^{ab}**	**76^{ab}**	**78^{a}**	**0.03**	**82^{a}**	**64^{b}**	**<.01**	76	70	0.07	0.15	0.40	2.434

[a,b,c] Means within a main effect with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
D x A = Interaction between dark exposure and age.
D x G = Interaction between dark exposure and gender.
No significant interactions between age and gender or between dark exposure, age and gender were observed.

Table 3.4. Effect of dark exposure, age, gender, and the interaction between dark exposure and age on length of feeding and drinking bouts during the photoperiod and scotoperiod of male and female broilers at weeks 2 and 4

	Dark Exposure (D)					Age (A, wk)			Gender (G)			D x A	SEM
	1	4	7	10	P value	2	4	P value	M	F	P value		
Photoperiod													
avg. feed bout length(s)	124	120	126	105	0.39	**103^{b}**	**135^{a}**	**<.01**	122	116	0.56	0.78	5.1
avg. drink bout length(s)	75	76	54	73	0.28	**61^{b}**	**79^{a}**	**0.05**	77	62	0.11	0.32	4.7
Scotoperiod													
avg. feed bout length (s)	**0^{b}**	**0^{b}**	**9^{b}**	**44^{a}**	**<.01**	**0.3^{b}**	**26^{a}**	**<.01**	11	15	0.56	**<.01**	6.2

^{a,b,c} Means within a main effect with common letters do not differ significantly (*P*≤0.05).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
G = Gender.
A = Age.
D x A = Interaction between dark exposure and age.
No significant interactions between dark exposure and gender, age and gender or between dark exposure, age and gender were observed.

Table 3.5. Effect of dark exposure, age, gender, and the interaction between dark exposure and age on interval between feeding and drinking bouts during the photoperiod and scotoperiod of male and female broilers at weeks 2 and 4

	Dark Exposure (D)					Age (A, wk)			Gender (G)			D x A	SEM
	1	4	7	10	P value	2	4	P value	M	F	P value		
Photoperiod													
avg. feed bout interval(s)	**1109**^{a}	**1070**^{ab}	**971**^{ab}	**827**^{b}	**0.03**	**865**^{b}	**1123**^{a}	**<.01**	**922**^{b}	**1066**^{a}	**0.04**	0.67	44.0
avg. drink bout interval(s)	**1338**^{a}	**1005**^{b}	**768**^{c}	**683**^{c}	**<.01**	**791**^{b}	**1106**^{a}	**<.01**	923	975	0.38	**0.04**	61.9
Scotoperiod													
avg. feed bout interval (s)	0	0	126	774	0.17	0	450	0.11	101	349	0.37	0.17	154.8

^{a,b,c} Means within a main effect with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
G = Gender.
A = Age.
D x A = Interaction between dark exposure and age.
No significant interactions between dark exposure and gender, age and gender or between dark exposure, age and gender were observed.

Table 3.6. Effect of dark exposure, age, gender, and the interaction between dark exposure and age on total time spent at the feeder and drinker during the photoperiod and scotoperiod of male and female broilers at weeks 2 and 4

	Dark Exposure (D)					Age (A, wk)			Gender (G)			DxA	SEM
	1	4	7	10	P value	2	4	P value	M	F	P value		
Photoperiod													
total time feeding (s)	**8742^{a}**	**7825^{ab}**	**6910^{ab}**	**6242^{b}**	**0.05**	7183	7676	0.43	8023	6836	0.07	0.79	322.3
total time drinking (s)	4601	5387	4079	5902	0.42	5020	4965	0.95	5782	4202	0.07	0.61	400.9
Scotoperiod													
total time feeding (s)	**0^{b}**	**0^{b}**	**19^{b}**	**212^{a}**	**0.01**	**0.3^{b}**	**115^{a}**	**0.03**	53	62	0.85	**0.01**	30.5

^{a,b,c} Means within a main effect with common letters do not differ significantly ($P \leq 0.05$).
SEM = Standard error of the mean.
D = Hours of dark over 24 h.
G = Gender.
A = Age.
D x A = Interaction between dark exposure and age.
No significant interactions between dark exposure and gender, age and gender or between dark exposure, age and gender were observed.

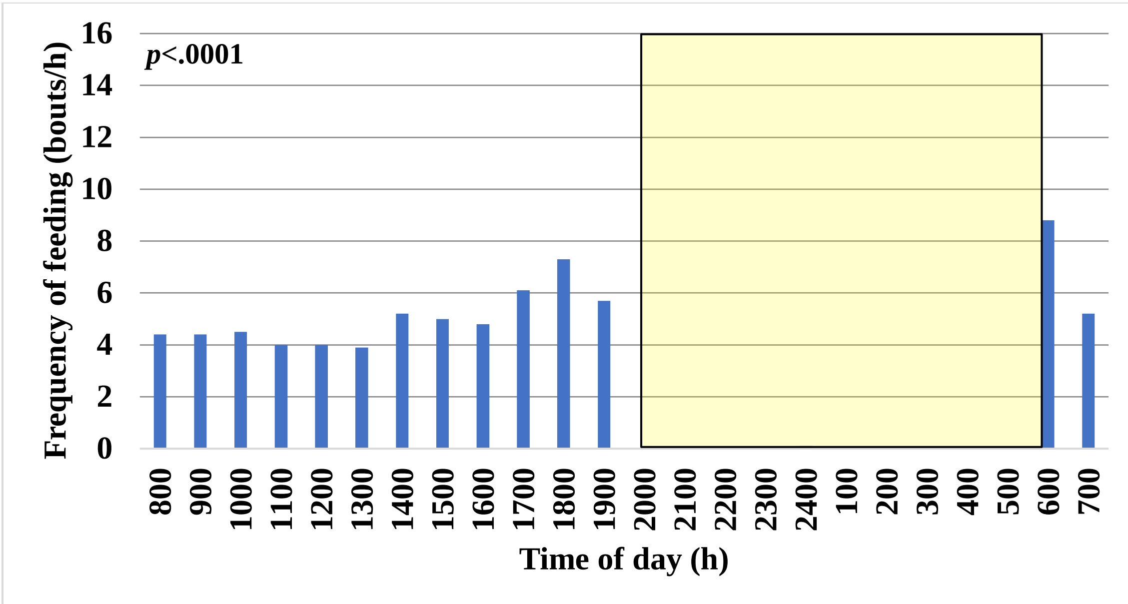

FIGURE 3.1. Frequency of feeding during the photoperiod and scotoperiod by 2 week old male broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

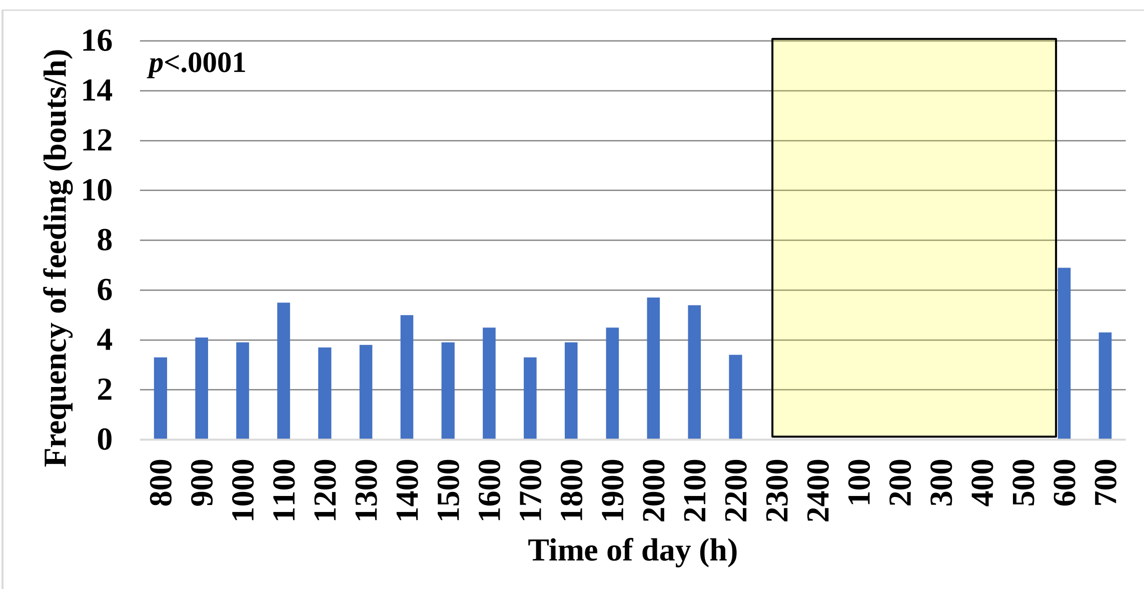

FIGURE 3.2. Frequency of feeding during the photoperiod and scotoperiod by 2 week old male broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

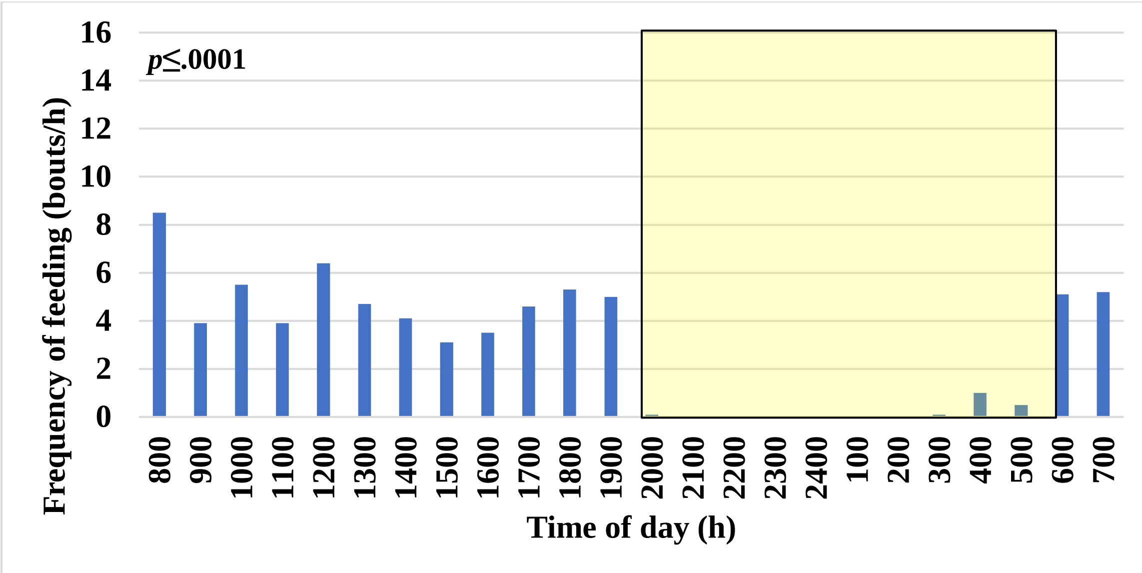

FIGURE 3.3. Frequency of feeding during the photoperiod and scotoperiod by 4 week old male broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

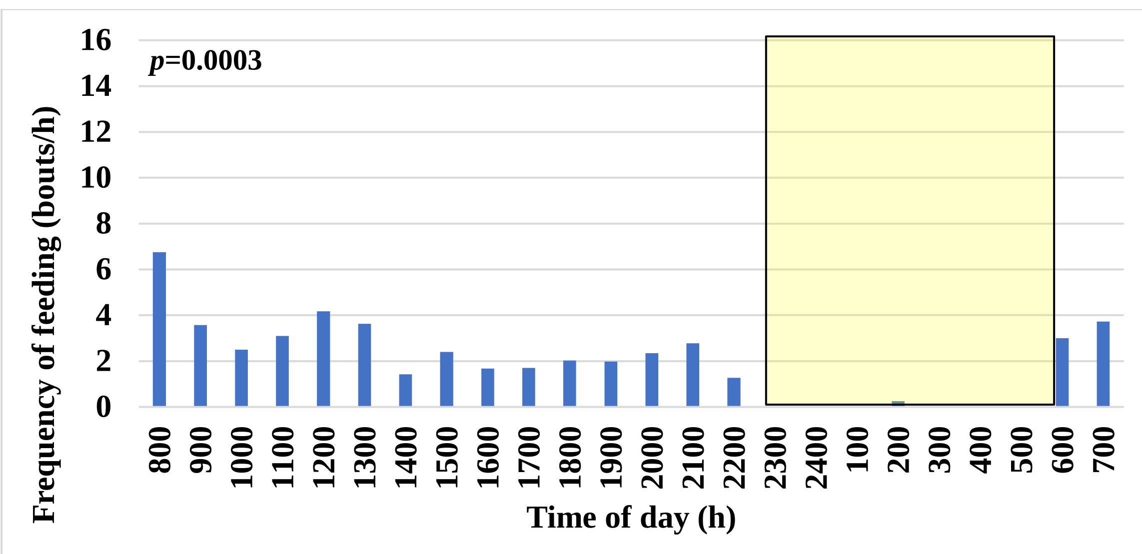

FIGURE 3.4. Frequency of feeding during the photoperiod and scotoperiod by 4 week old male broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

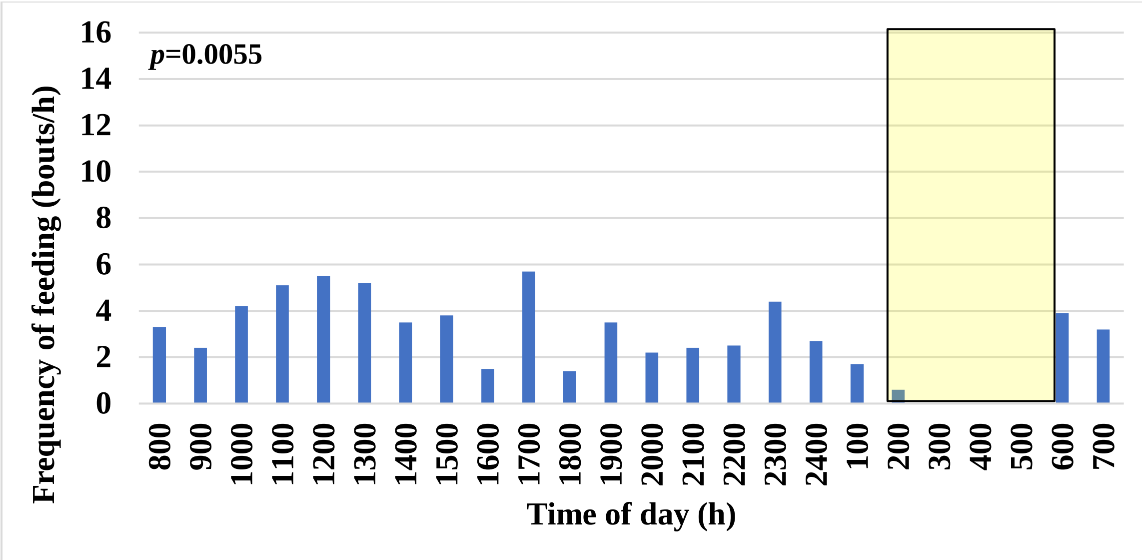

FIGURE 3.5. Frequency of feeding during the photoperiod and scotoperiod by 4 week old male broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

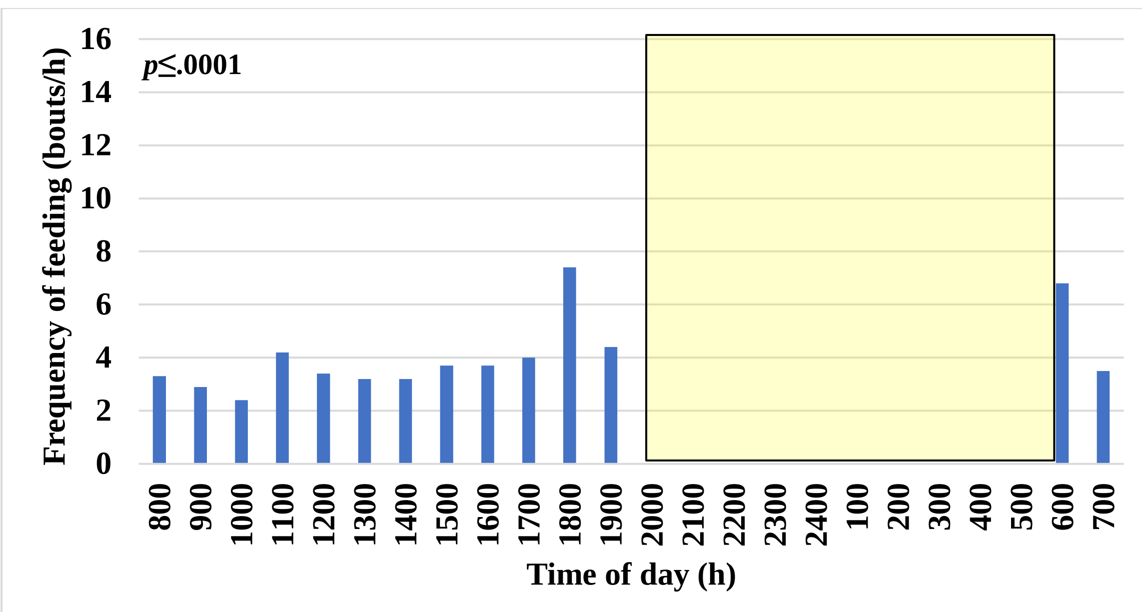

FIGURE 3.6. Frequency of feeding during the photoperiod and scotoperiod by 2 week old female broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

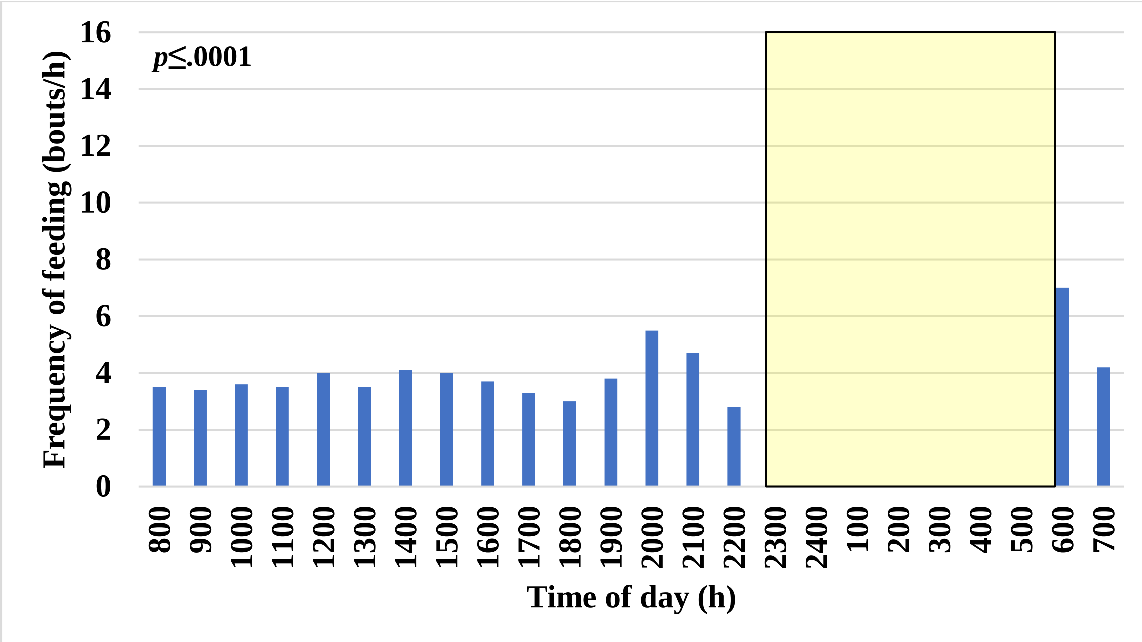

FIGURE 3.7. Frequency of feeding during the photoperiod and scotoperiod by 2 week old female broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

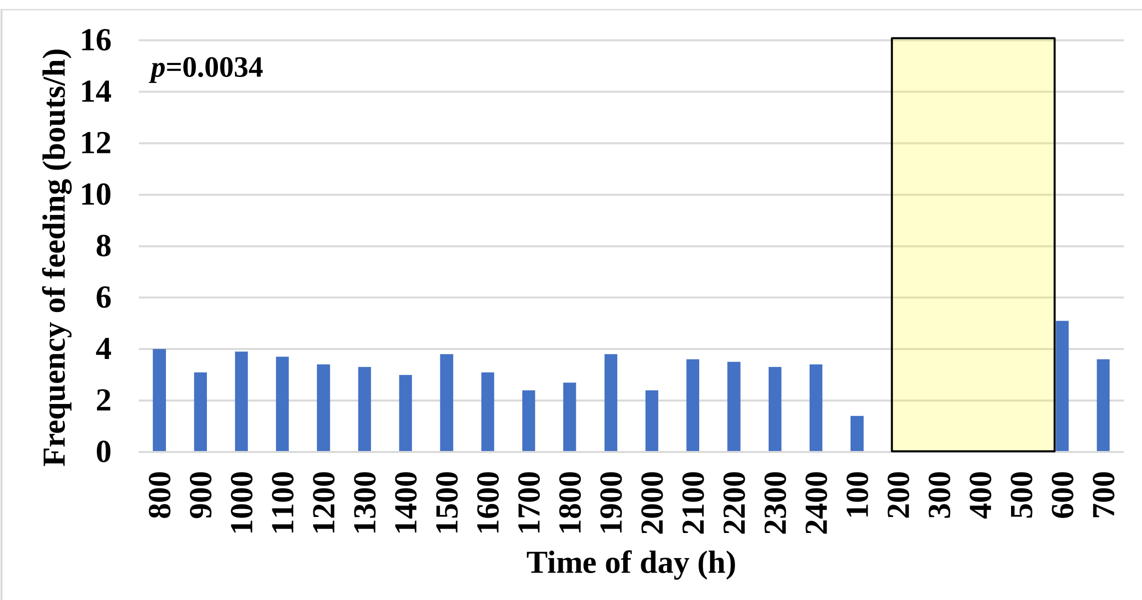

FIGURE 3.8. Frequency of feeding during the photoperiod and scotoperiod by 2 week old female broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

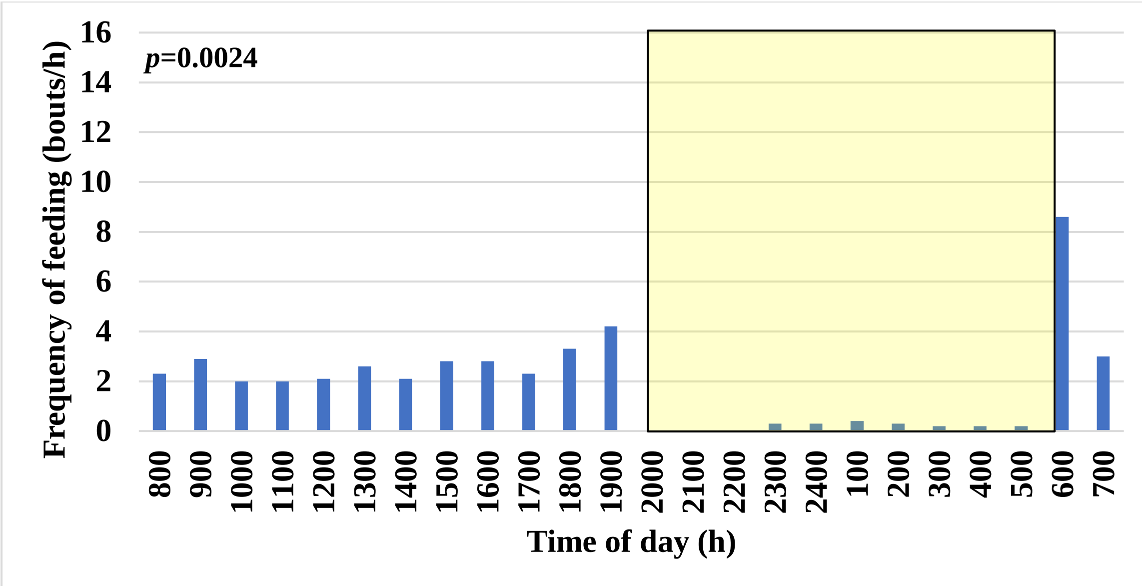

FIGURE 3.9. Frequency of feeding during the photoperiod and scotoperiod by 4 week old female broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

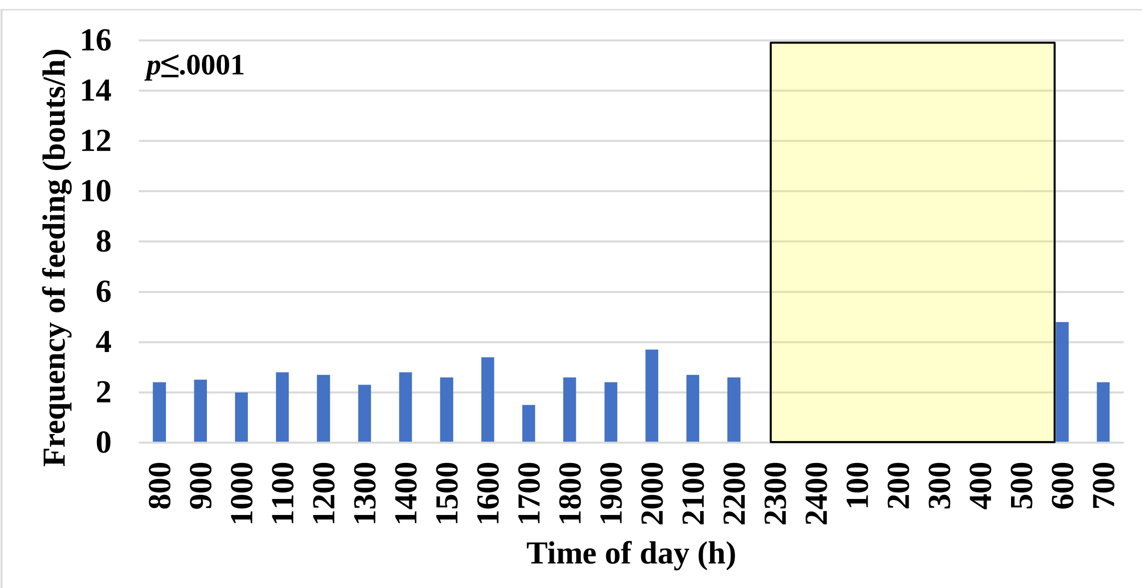

FIGURE 3.10. Frequency of feeding during the photoperiod and scotoperiod by 4 week old female broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

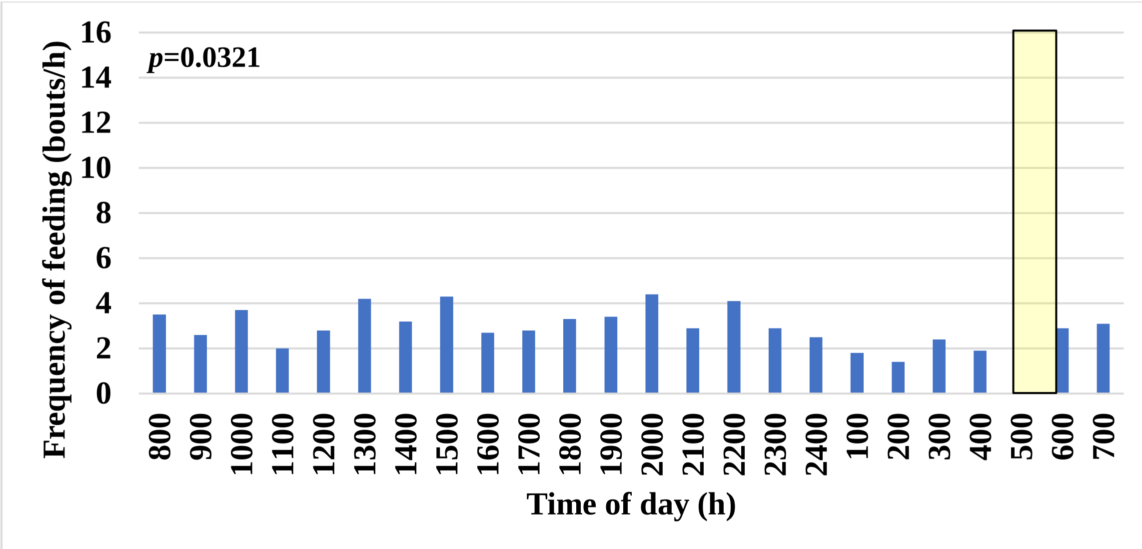

FIGURE 3.11. Frequency of feeding during the photoperiod and scotoperiod by 4 week old female broilers exposed to 1 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response during the photoperiod.

4.0 Chapter 4: Overall Discussion

4.1 Introduction

A number of recommended practices for boiler lighting programs exist (for example, Primary Breeders world-wide, Codes of Practice in Canada and European legislation), with no universal agreement on how many hours of darkness to provide. Economics are driven by bird performance and therefore lighting programs are often selected based on their impact on productivity, with less focus on bird welfare.

Attitudes with regards to the importance of dark exposure for broilers are changing and this has been reflected in updated regulations in some countries. Canada has recently updated its requirement for photoperiod duration (NFACC, 2016). The National Farm Animal Care Council's current Codes of Practice for hatching eggs, breeders, chickens and turkeys require producers to gradually increase the amount of darkness from 0 to 4 h, per 24 h period, by day 5 and maintain this minimum level of darkness until at least 7 days prior to catching (NFACC, 2016). This is an improvement from the previous requirement of only 1 h of darkness per day (NFACC, 2016), but the new requirements for duration of darkness in Canada are still lower than other countries. Legislation from the European Union requires producers to provide, at minimum, a total of 6 h of darkness in every 24 h period, with at least 4 h of continuous darkness, after the first 7 d until 3 d before slaughter for broilers (European Commission, 2007).

In order for new recommendations to be made, it is important to conduct research that investigates the implications of various management practices on a number of parameters. The purpose of the study was to enhance the current understanding of how behaviour is altered by lighting program and how these changes relate to observed differences in productivity and gastrointestinal tract segment and content weights.

4.2 Objectives

The primary objectives of this work were to determine if duration of darkness had an impact on the feeding behaviour of commercial broilers and whether duration of darkness and the resulting behavioural adaptations affect the size and content of the gastrointestinal tract segments. Secondary objectives were to determine the effect of bird age and gender on feeding and drinking behaviour. Graded levels of darkness were used to allow for regression analyses to examine the relationships between dark exposure and the dependent variables. The lighting programs used were 23L:1D, 20L:4D, 17L:7D and 14L:10D.

4.3 Productivity

Historically it was believed that constant and near-constant lighting programs provided the most visual access to feed and thus should have allowed for the highest feed intake and fastest growth rate resulting in the highest market body weights. The current study used graded levels of darkness (1D, 4D, 7D, and 10D), to examine the relationship between dark exposure and productivity. The data shows that birds raised to 31d, with only 1 h of darkness, do not achieve the highest body weights. Instead, the highest final body weights were achieved by birds reared under 4 and 7 h of darkness. This could be due to a number of reasons, for example, providing a dark period alters the timing of a bird's growth curve by reducing growth rate early in life. Additionally, birds learn to anticipate the dark periods and change their feeding behaviour to accommodate the period without feed, which may improve digestibility. Finally, although not demonstrated in this work, the darkness itself is beneficial in terms of reducing skeletal and metabolic disease, improving immune function and improving the quantity and quality of sleep. Very long dark periods (10 h in this work) result in lower feed consumption for broilers, but using up to 7 h of darkness does not impact the ability of birds to consume feed. After examining the body weight and feed consumption data it is not surprising that feed efficiency was shown to improve with increasing levels of darkness. This may be due to a number of factors including increased melatonin production and reduced energy expenditure during the dark period (Apeldoorn et al., 1999).

4.4 Gastrointestinal tract segment and content weights

Providing birds with a dark period also affects the storage of feed in the gastrointestinal tract, which likely impacts feed passage rate. In birds reared with dark periods of 4 h or more, an increase in feeding activity occurs prior to the beginning of the scotoperiod, which indicates that birds have learned to anticipate the onset of these dark periods. The purpose of this anticipatory increase in feed consumption is to maintain a source of energy throughout the coming dark period. No such increase in activity and consumption was observed in birds reared on near-continuous lighting and it has been suggested that a minimum of four continuous hours of darkness is necessary to induce this behaviour (Buyse et al., 1993), which was confirmed by this study. The average retention time in the digestive tract, excluding the ceca, is 4 to 8 h in broiler chickens raised on continuous light (Hetland and Svihus, 2001). Previous research has shown that feed transit time is longer during the scotoperiod (Buyse et al., 1993). Cutler et al. (2005) showed that turkeys raised on 14L:10D had ingesta present in their crops for up to 9 h after the end of the photoperiod. Results of the present study show that, despite varying hours of darkness in which birds typically do not eat, feed is still present in the gastrointestinal tract after 10 h of darkness. A slower feed transit time is indicative of a longer retention time, which may result in an improvement in digestibility. Therefore, it is possible that the improvement in production parameters including body weight and feed efficiency could be partially explained by an improvement in nutrient digestibility in birds reared under dark periods. The crop and gizzard represent the locations where feed transit time is likely effected the most. The longer that feed remains in the crop, the more time it has to be exposed to moisture and enzymes, increasing the surface area of the ingesta. A subsequent increase in the time spent in the gizzard allows for more mechanical and chemical degradation of the ingesta, again increasing surface area. This should lead to an improvement in digestibility and nutrient absorption in the small intestine.

Exposure to darkness also affects the size of the gastrointestinal tract segments, which reflects their utilization and possibly their functioning. The observed increase in crop size with increasing levels of darkness is due to the increased utilization of the crop as a storage organ. The longer the dark period, the more feed that needs to be stored to maintain a source of energy for the duration of the dark period. The crop has a thin membrane like structure that is well innervated and vascularized. With more utilization the crop distends and increases in musculature, leading to an increase in crop size and weight. Longer dark periods also result in an increase in gizzard weight. The gizzard is a muscular compartment and an increase in utilization results in more contractions and an increase in musculature, which may in turn explain the increase in weight. The gizzard plays a very small role in feed storage compared to the crop, however it plays a very important role in regulating the passage of feed throughout the gastrointestinal tract. An increase in the contents of the crop and gizzard due to anticipatory feeding before a dark period requires an increase in gizzard activity and therefore an increase in muscle mass. An increase in musculature suggest a longer feed residency time in the gizzard, which may result in a greater degree of mechanical grinding of feed and exposure to acid from the proventriculus, leading to an improvement in digestibility or feed efficiency. Buyse et al. (1993) estimated that the storage of feed in the anterior digestive tract along with the longer feed transit time (measured using ferric and chromic oxide) during the scotoperiod was responsible for 75.5% of the bird's nocturnal energy needs. It was also found that total heat production during the scotoperiod amounted to less than half of the total heat production during the photoperiod. Therefore maintenance energy requirements would be lower during the dark period, resulting in energy conservation. This could help to explain the increased feed efficiency observed in birds reared on longer dark periods.

The increased utilization of the crop, with increasing dark periods, may also have an effect on GIT health. With larger quantities of feed being stored in the crop, as well as a longer retention time, this promotes the colonization of beneficial bacterial species, especially of the Lactobacillus variety. These lactobacilli produce organic acids which allows for microbial fermentation to occur thus reducing the pH of the crop. This could lead to improved chemical and mechanical digestion later in the digestive tract and therefore improved nutrient absorption in the small intestine. Another benefit of a large and stable population of lactobacilli is the inhibition of colonization of harmful bacterial species such as Salmonella. Reduced pH in the crop as well as increased exposure of digesta to acid as a result of increased gizzard functionality may also enhance the acid barrier function of the gastrointestinal tract. It is also possible that an improvement in digestibility could reduce the substrate available to bacteria in the lower GIT, again suggesting a possible impact on GIT health.

4.5 Behaviour

Examining behaviour is a useful tool to assess bird welfare, which is demonstrated by including expression of normal behaviour as one of the Five Freedoms. It has been observed that birds reared on constant or near-constant lighting programs were easier to catch during loadout. It is likely that this change in behaviour is due to birds being more lethargic and sleep deprived when not given enough access to dark periods that enable them to achieve an improved quantity and quality of sleep/rest. In the current work, very little feeding and no drinking activity was observed during the dark periods, which could suggest that birds are using this period to rest/sleep. Although not reported in this study, the observer noticed that birds spent the majority of the dark period lying down. It is also possible that birds raised with long photoperiods have impaired skeletal health due to their rapid growth rate, especially early in life when their skeletal structure is still developing, resulting in a large body weight resting on a compromised frame. As was discussed previously in the productivity section, providing birds a dark period allows for slower early growth, which may improve skeletal integrity. These explanations may also apply to the changes observed in constant and near-constant light reared birds who show reduced overall activity and reduced mobility behaviours.

Results of this study show that birds raised on longer dark periods visit both the feeder and drinker more often, which indicates that these birds are more active than birds raised on short dark periods. This may increase behavioural pattern complexity (Sinclair et al., 2015), which refers to the average number of behaviours within a pattern. Increases in complexity indicate that performance of a behaviour (or general activity) has increased or that intervals between behaviours has become less variable (Sinclair et al., 2015). A reduction in complexity may indicate an impaired state, such as stress or disease (MacIntosh et al., 2011).

In addition to behavioural output being used as a tool to aid in assessing bird welfare, the study of behavioural patterns may also help to understand changes in production parameters, as demonstrated by the data and conclusions drawn in this work. Broilers in today's industry have been heavily selected for increased body weight and are highly motivated to feed. As the duration of darkness that birds are exposed to increases, their visits to the feeder become more frequent and intervals between feeding become shorter. This is interesting because the birds reared with 1, 4, and 7 hours of darkness spent the same amount of time at the feeder and consumed the same amount of feed. Therefore, this change in behaviour, observed in birds on moderate dark periods, is not to increase feed consumption, but may be more related to pattern of feed intake or better mobility due to improved brain functioning and/or improved walking ability. However it is possible that the longest dark period (10 h) caused birds to increase their feeding to compensate for the shorter photoperiod available to consume feed. If this is the case then 10 hours of darkness appears to present enough of a challenge that birds increase the frequency of their feeding and retain more feed in their crops throughout the day. However, it is not enough of a challenge to cause them to consume a substantial amount of feed during the dark.

In this thesis, studying behaviour also helped to explain changes in the contents of the gastrointestinal tract of birds reared on different durations of darkness. Birds were able to consume enough feed prior to a 10 h dark period to still have ingesta remaining in the ileum at the end of that dark period. Broilers undoubtedly learned to anticipate that darkness was coming when exposed to at least 4 h of continuous darkness. In order to avoid having any sections of the GIT empty for long periods during the dark (research on feed withdrawal in broilers shows that GIT integrity declines significantly with long periods of withdrawal (Thompson and Applegate, 2006), birds increase the number of feeding bouts prior to lights turning off. In fact, this resulted in feed remaining present throughout the gastrointestinal tract until lights turned on even after 10 h of continuous darkness.

4.6 Conclusions

Duration of dark exposure has an impact on broiler productivity, gastrointestinal tract segment and content weights, and feeding behaviour. The 23L:1D program used in this study resulted in a reduction in bird performance. It was also associated with changes in bird behaviour including reduced feeding activity and lack of a diurnal feeding rhythm. This change in behavioural expression could be an indicator of reduced welfare when referring to the Five Freedoms, which stipulate that an animal should have the ability to express normal behaviours. Providing birds with a dark period resulted in improved productivity and a change in feeding behaviour that affected gastrointestinal tract size and contents. This impact of dark exposure on the GIT may have beneficial effects on digestibility, feed efficiency and gastrointestinal tract health.

Body weight was maximized under moderate dark periods of 4D and 7D. A decrease in feed intake was observed under 10D, but this lighting program resulted in the highest feed efficiency. The highest mortalities were found under 4D and 7D, however previous work with larger sample sizes and more replicates show lower mortalities as duration of darkness increases.

The behaviour data show that adding darkness to a lighting program increases feeding pattern complexity. Birds with longer dark periods have the highest number of bouts and the shortest intervals between bouts with no change in bout length. This indicates that these birds are more active and mobile and that they may have to eat more often to meet their feed requirements. Looking at the total time spent at the feeder in conjunction with the body weight and feed intake data is very informative. Birds reared on 1D are among those who spent the most total time at the feeder, however they do not consume more feed than birds raised with 4D or 7D and do not weigh more than these birds either. Conversely, birds reared with 10D spent less time at the feeder, consumed the least amount of feed and weighed the same as birds raised with 1D. Comparing the results obtained in this study to studies by Schwean-Lardner et al. (2012a, 2012b, 2014) which did not differ greatly in lighting program, rearing environment, diet or bird strain and age, but included more replications resulted in similar conclusions for body weight, feed consumption and feed efficiency. Schwean-Larder et al. (2012b) found that increasing the duration of darkness resulted in lower flock mortality, whereas we did not, likely due to a limited number of repetitions. In terms of feeding behaviour, Schwean-Lardner et al. (2012a) found that birds on longer dark periods spent a higher percent of time at the feeder than birds reared on shorter dark periods, which is contradictory to the results obtained in this study. This difference could be due to differences in behavioural assessment (scan vs. focal sampling). It is also possible that in the time between studies, the bird’s feeding behaviour has changed through genetic selection for increased feed intake.

In conclusion, the data collected in this thesis project provide a better understanding of how duration of darkness affects the feeding behaviour of broilers. It also illustrates how birds are able to adapt to their environments and how this in turn relates to observed changes in the gastrointestinal tract as well as production traits. Increasing the length of darkness that birds are exposed to leads to an increase in their behavioural pattern complexity, shown by an increase in the frequency of feeding. This in turn allows birds to adapt and increase their feed consumption prior to darkness, which maintains ingesta content in the gastrointestinal tract throughout the dark period.

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6.0 Appendices

Appendix A: Empty gastrointestinal tract segment weights and content weights over 24 h for each lighting program

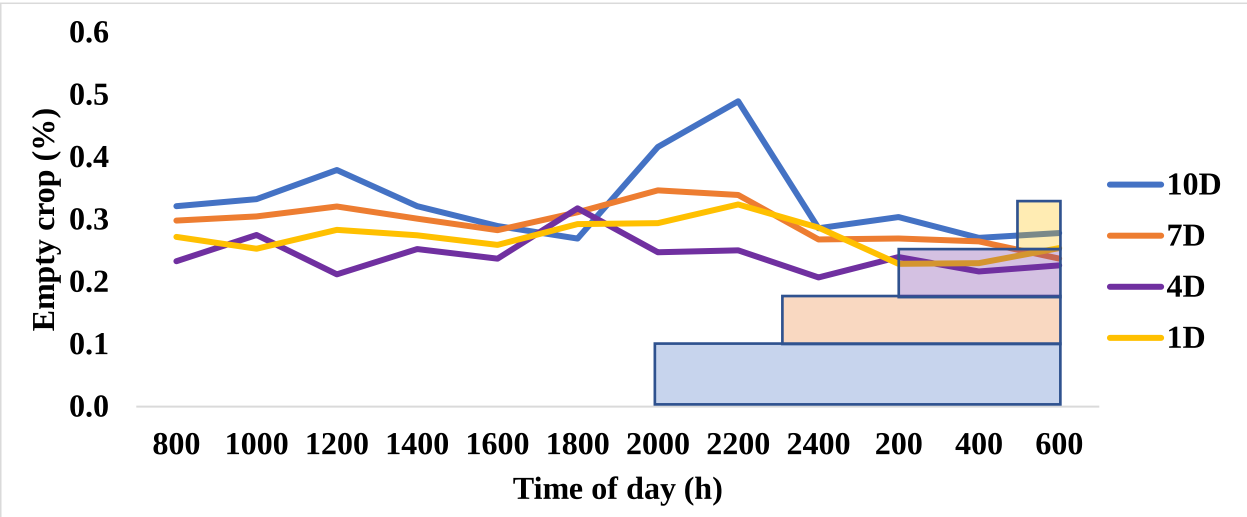

FIGURE A1. Effect of time and dark exposure on empty crop expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

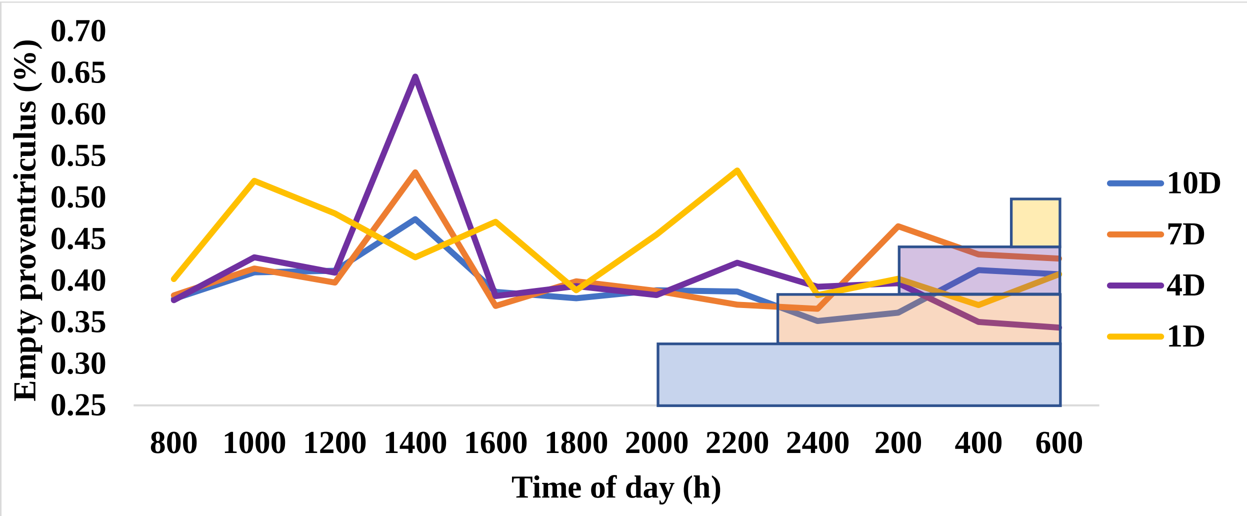

FIGURE A2. Effect of time and dark exposure on empty proventriculus expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

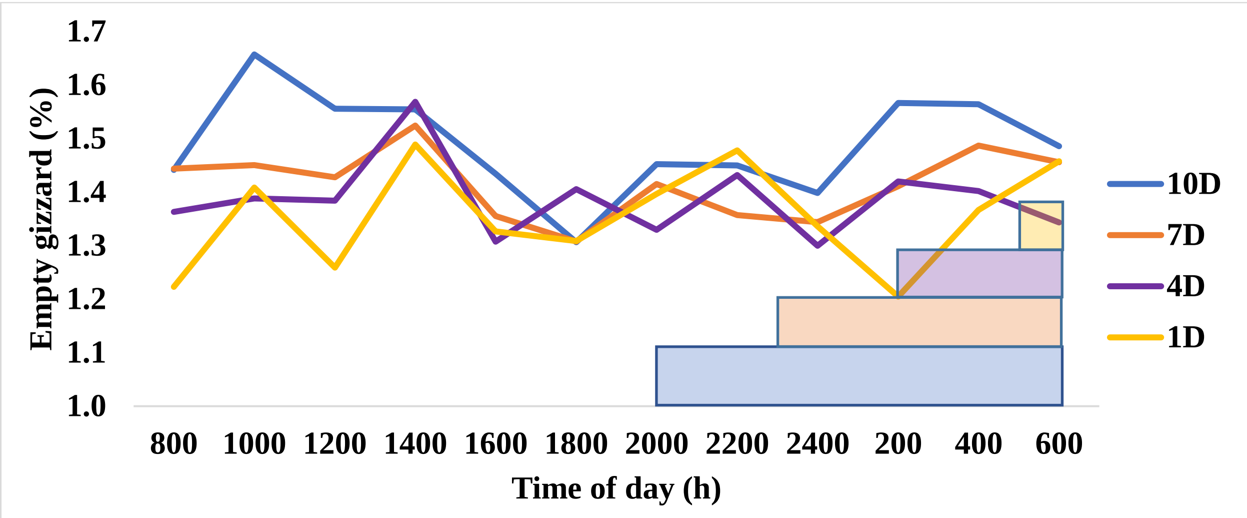

FIGURE A3. Effect of time and dark exposure on empty gizzard expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

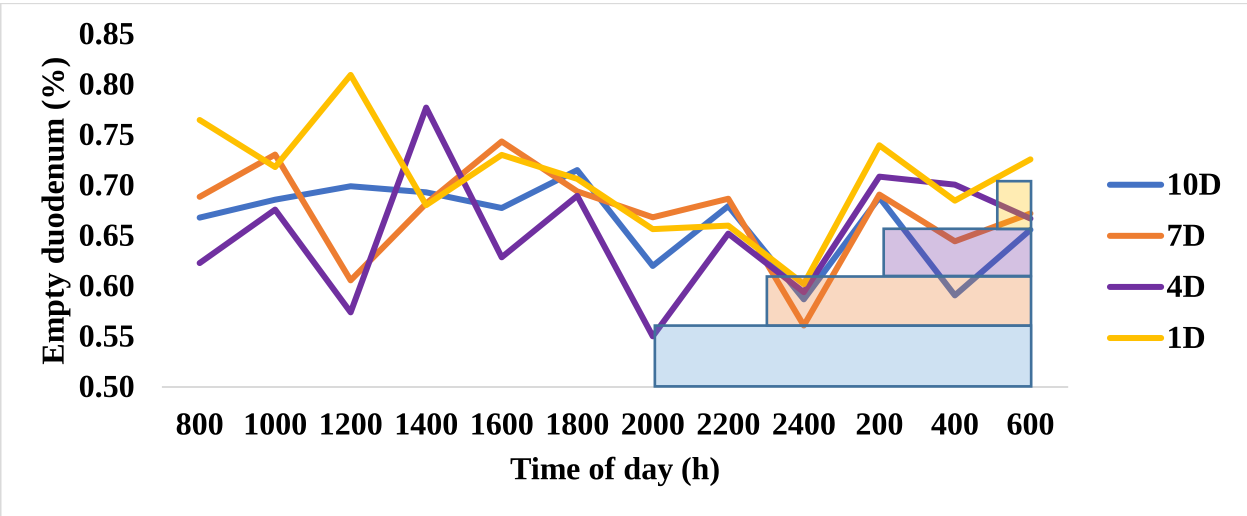

FIGURE A4. Effect of time and dark exposure on empty duodenum expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

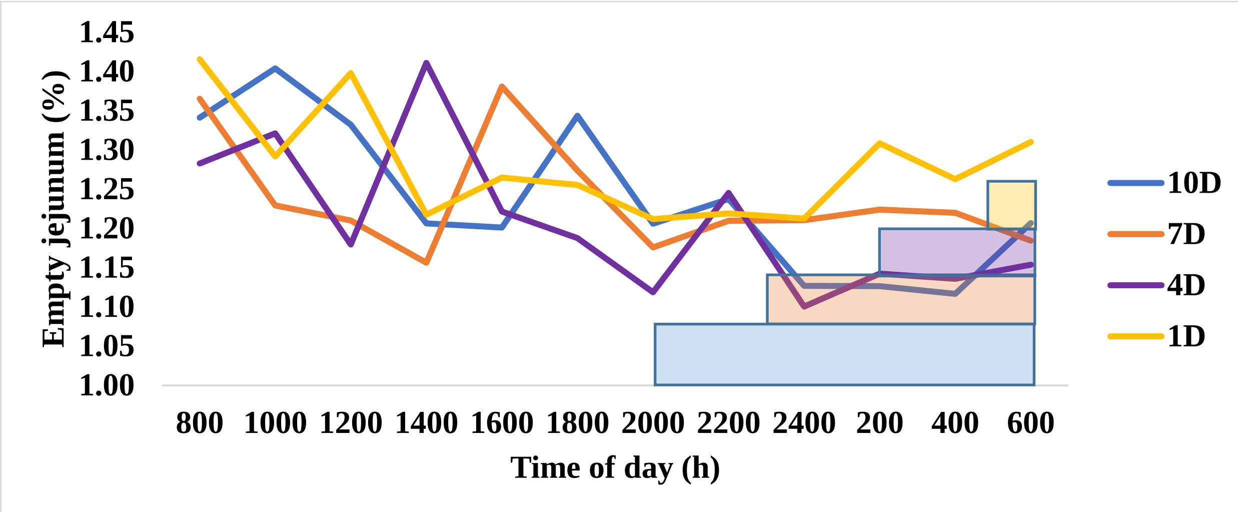

FIGURE A5. Effect of time and dark exposure on empty jejunum expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

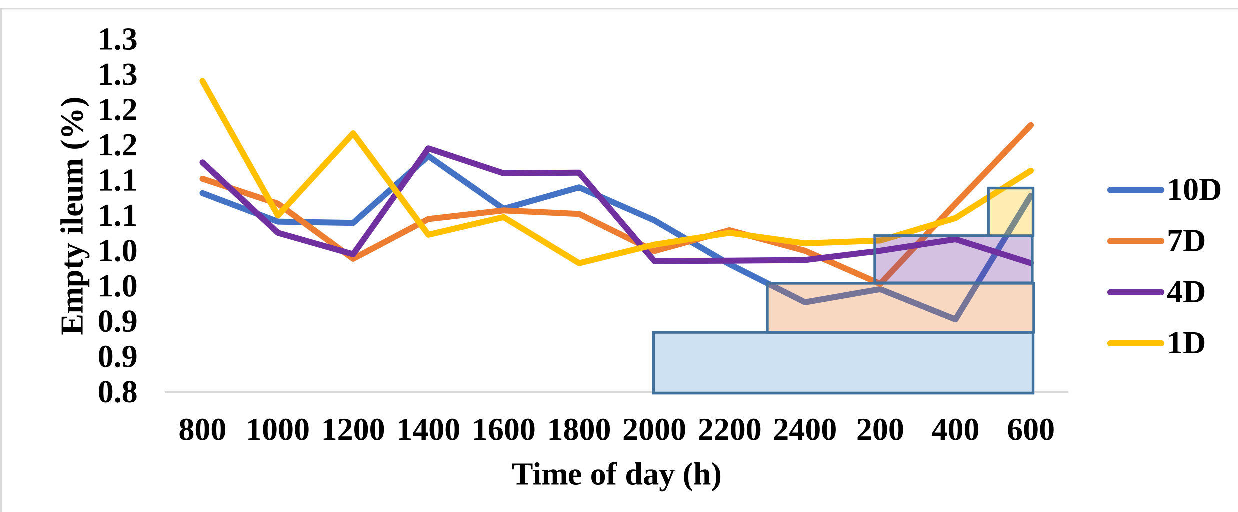

FIGURE A6. Effect of time and dark exposure on empty ileum expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

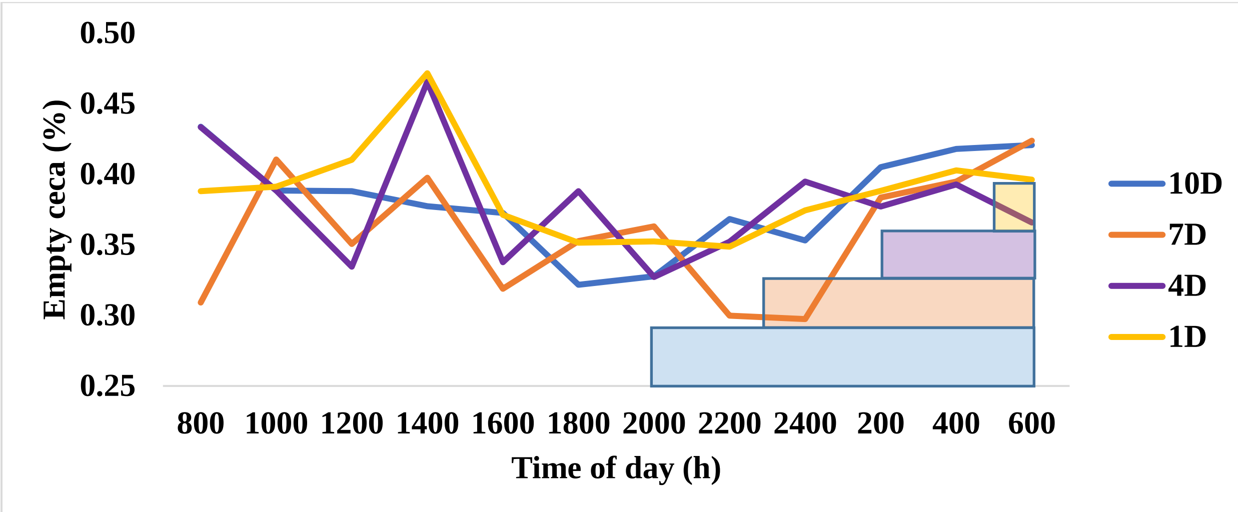

FIGURE A7. Effect of time and dark exposure on empty ceca expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

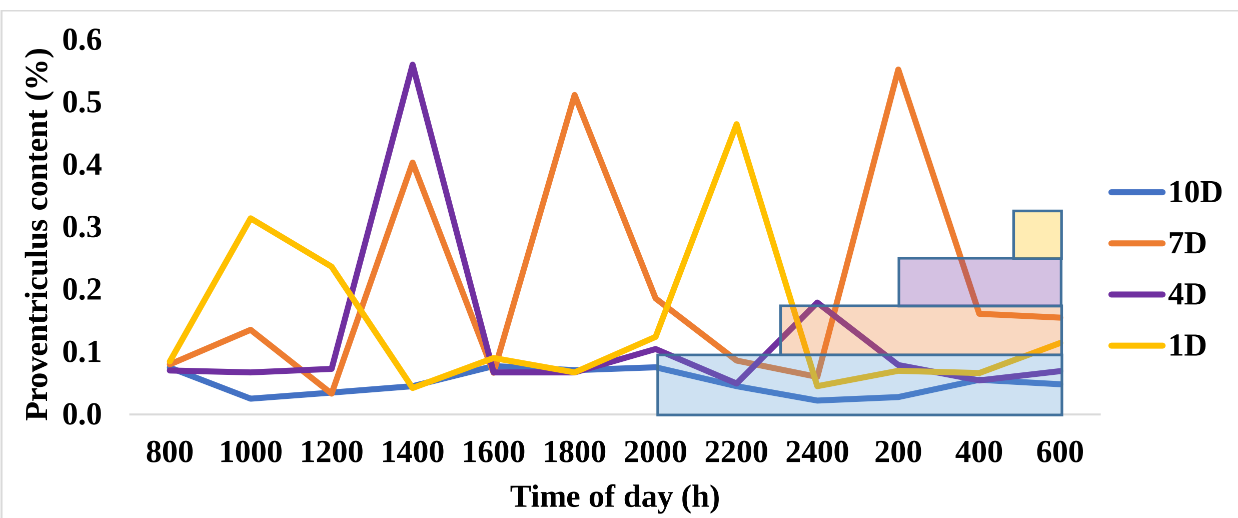

FIGURE A8. Effect of time and dark exposure on proventriculus content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

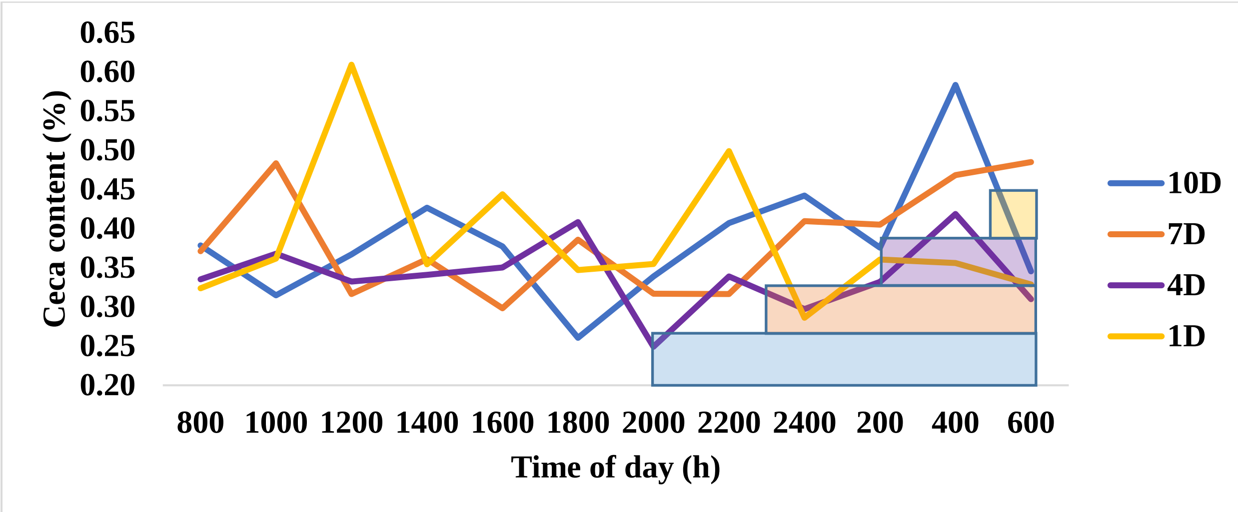

FIGURE A9. Effect of time and dark exposure on ceca content expressed as a percentage of body weight. The shaded boxes represent each corresponding scotoperiod, with the photoperiod resuming at 0600 for each lighting program.

Appendix B: Frequency of nutritive bouts for male and female broilers at 2 and 4 wk

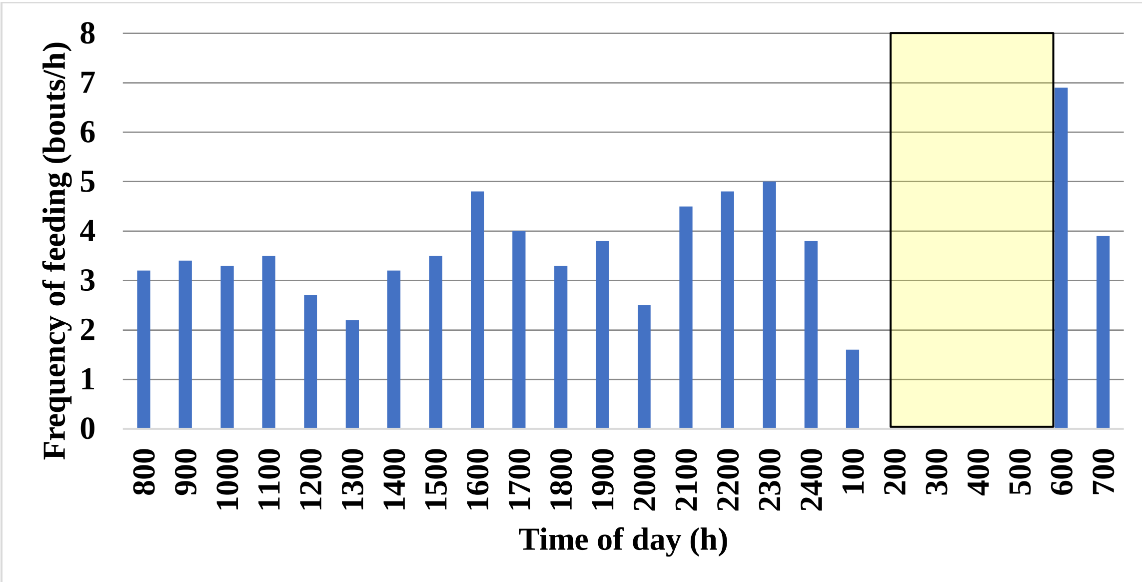

FIGURE B1. Frequency of feeding during the photoperiod and scotoperiod by 2 week old male broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod.

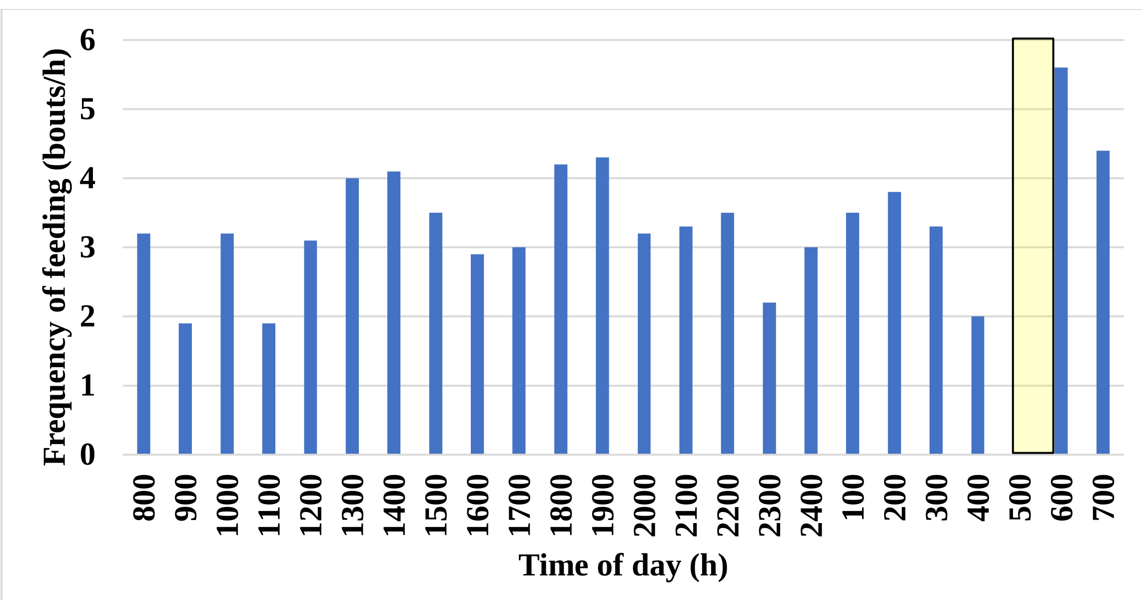

FIGURE B2. Frequency of feeding during the photoperiod and scotoperiod by 2 week old male broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

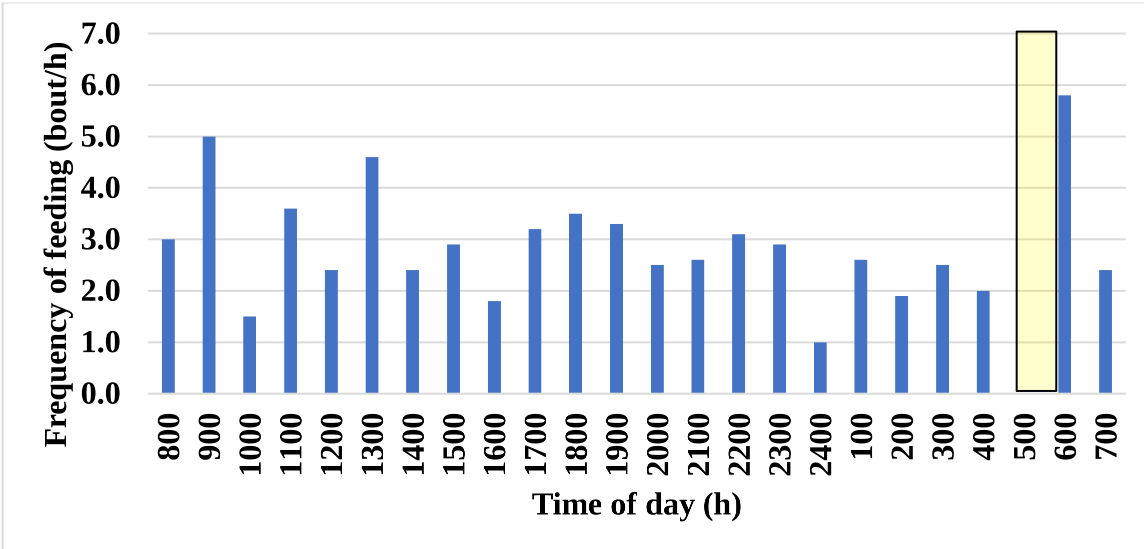

FIGURE B3. Frequency of feeding during the photoperiod and scotoperiod by 4 week old male broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

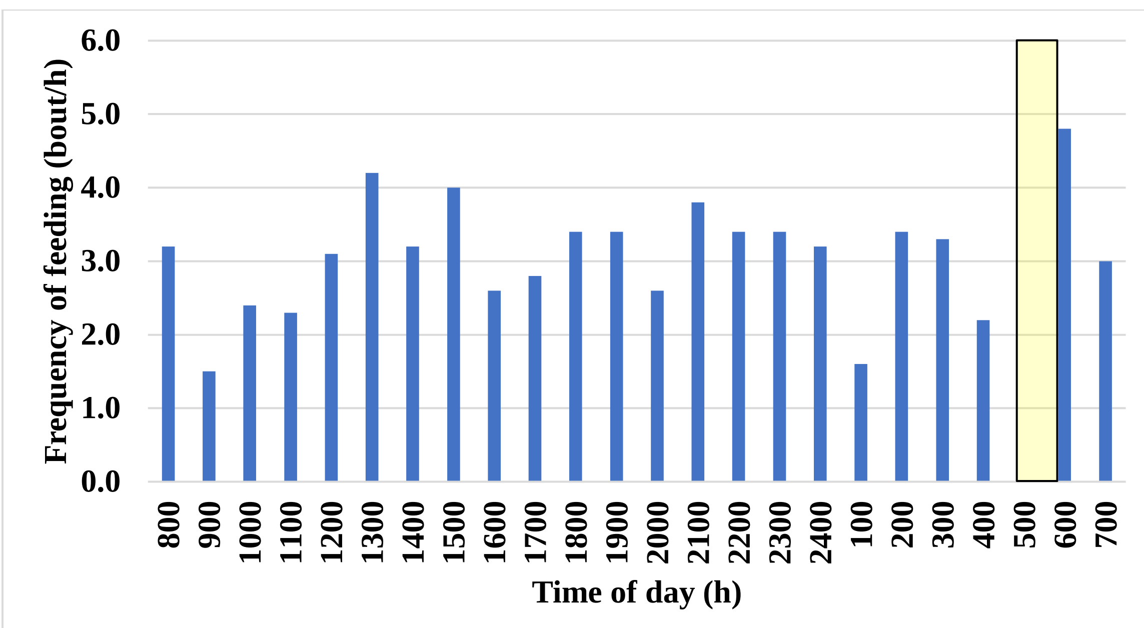

FIGURE B4. Frequency of feeding during the photoperiod and scotoperiod by 2 week old female broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

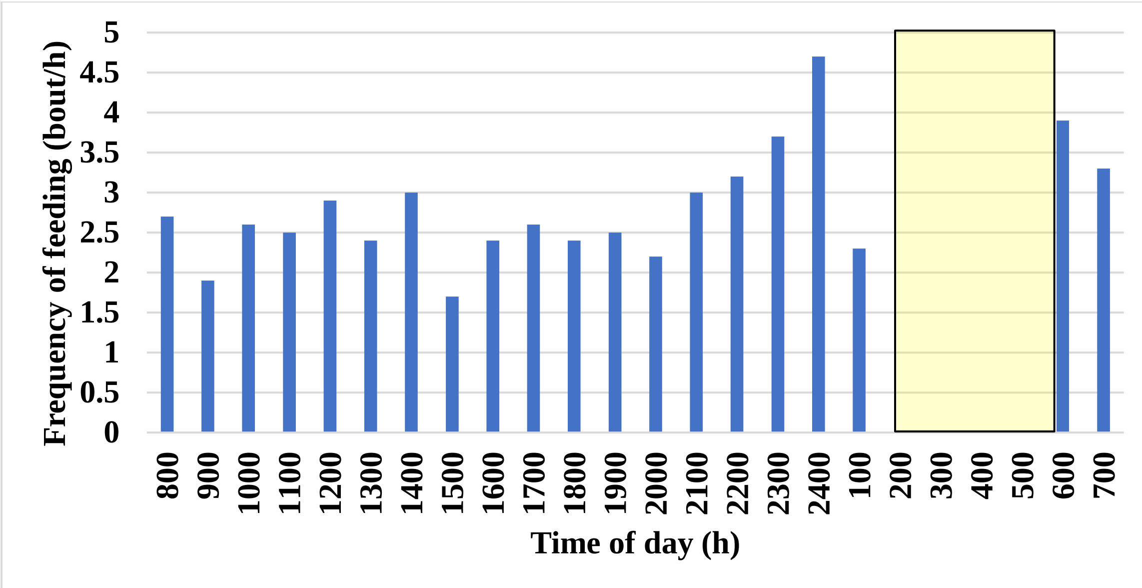

FIGURE B5. Frequency of feeding during the photoperiod and scotoperiod by 4 week old female broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod.

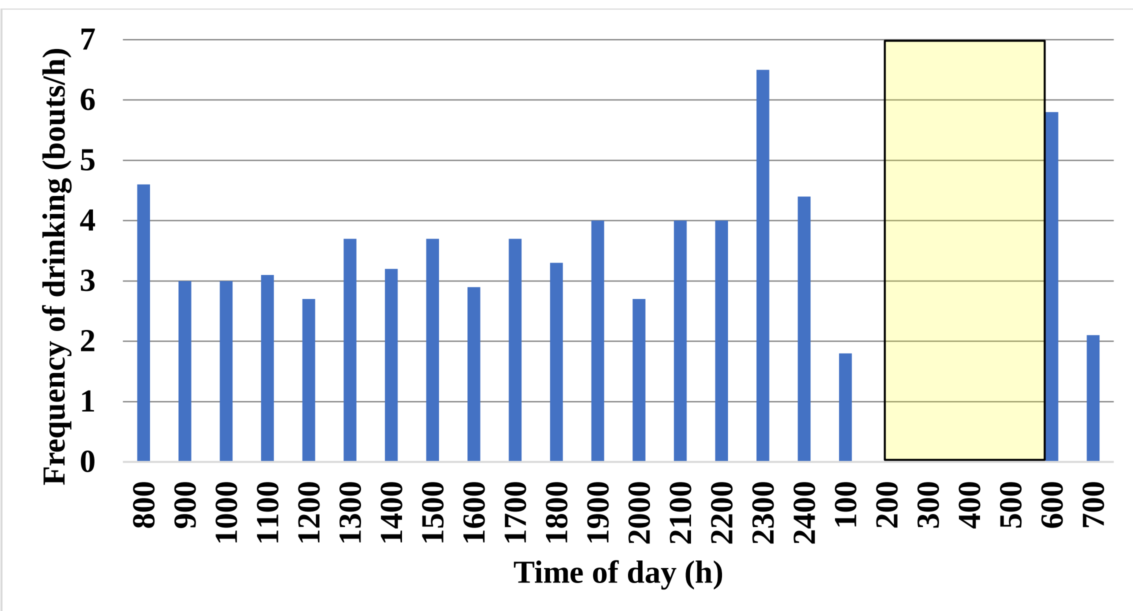

FIGURE B6. Frequency of drinking during the photoperiod and scotoperiod by 2 week old male broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod.

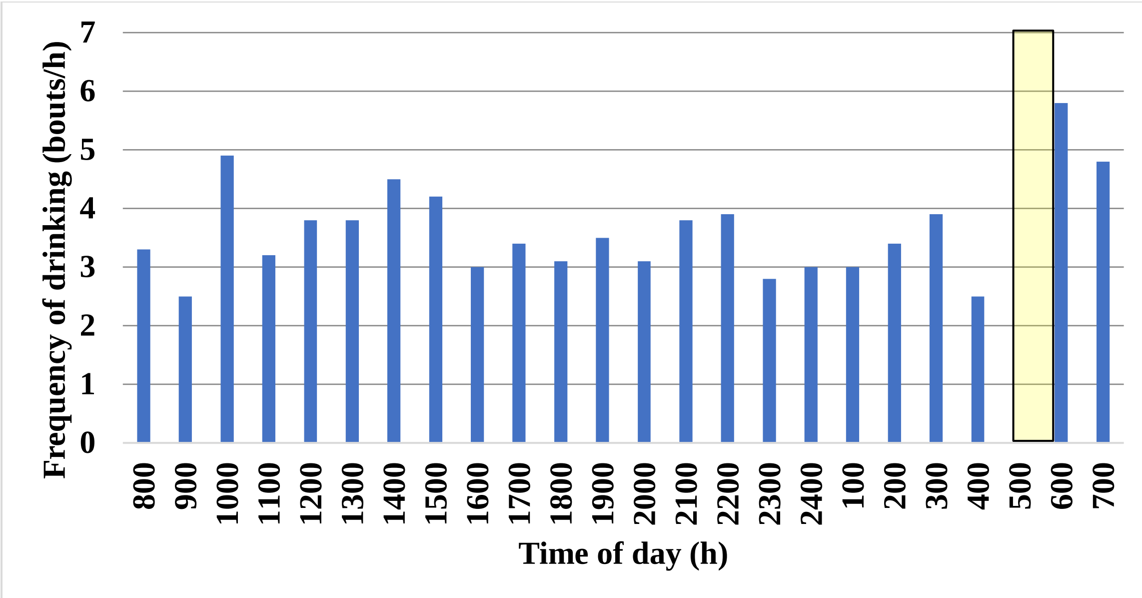

FIGURE B7. Frequency of drinking during the photoperiod and scotoperiod by 2 week old male broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

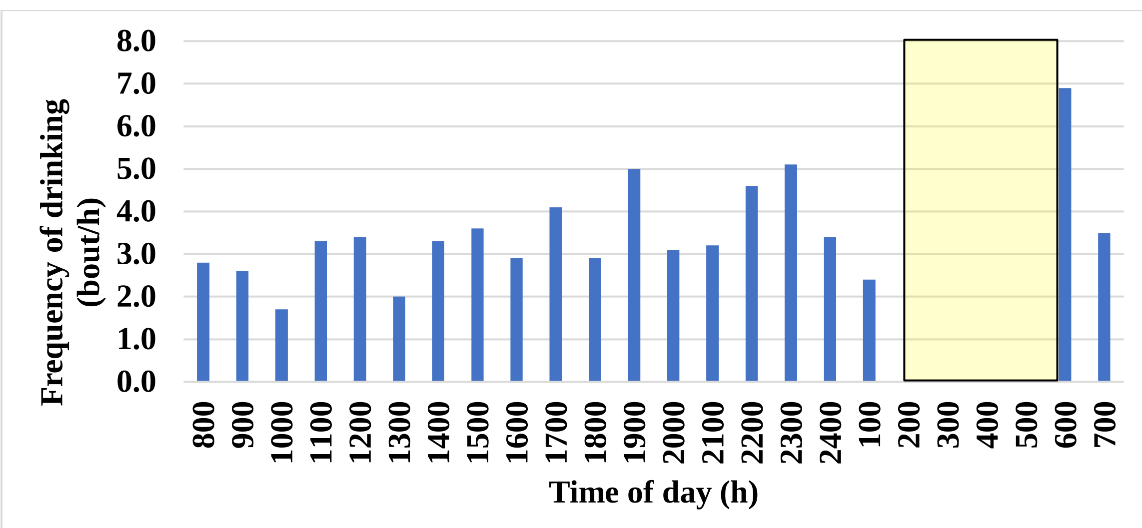

FIGURE B8. Frequency of drinking during the photoperiod and scotoperiod by 4 week old male broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod.

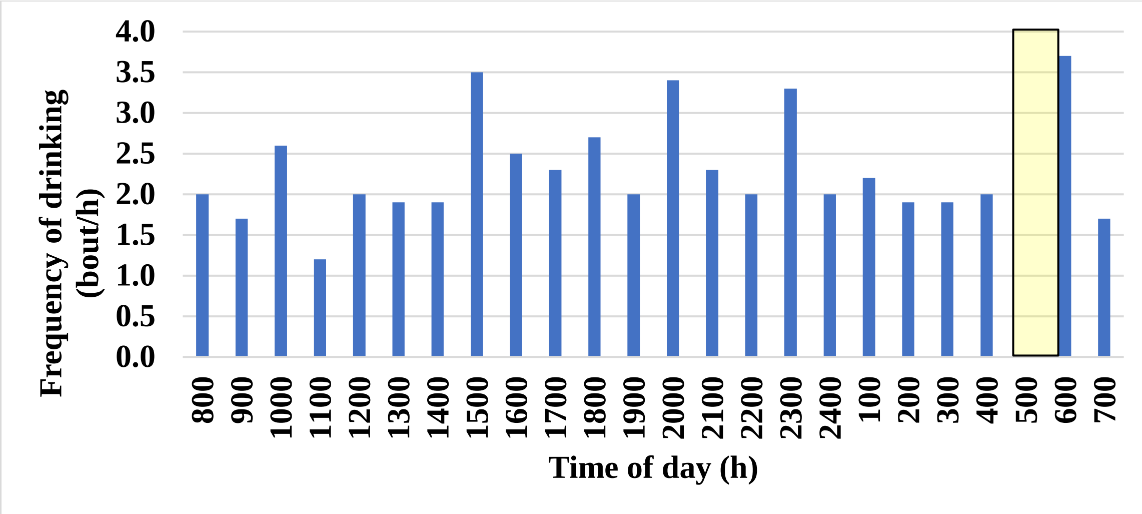

FIGURE B9. Frequency of drinking during the photoperiod and scotoperiod by 4 week old male broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

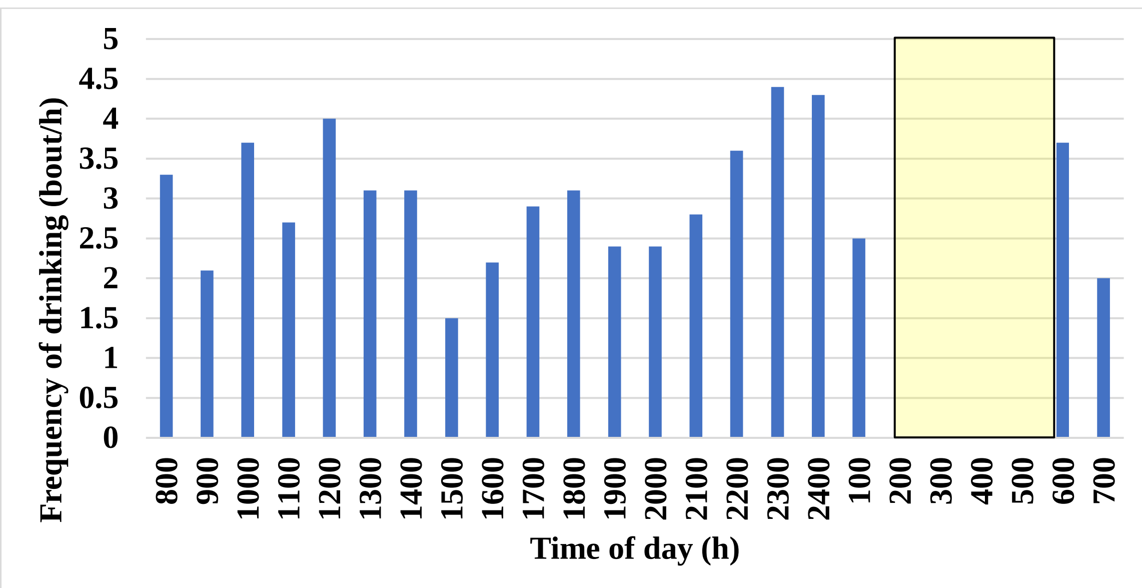

FIGURE B10. Frequency of drinking during the photoperiod and scotoperiod by 4 week old female broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod.

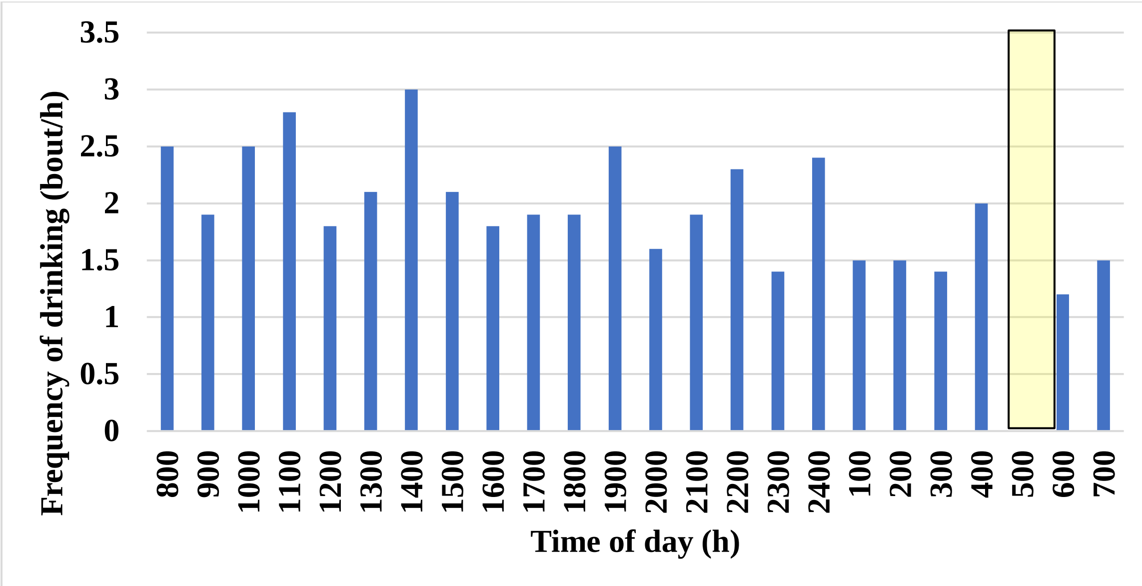

FIGURE B11. Frequency of drinking during the photoperiod and scotoperiod by 4wk old female broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod.

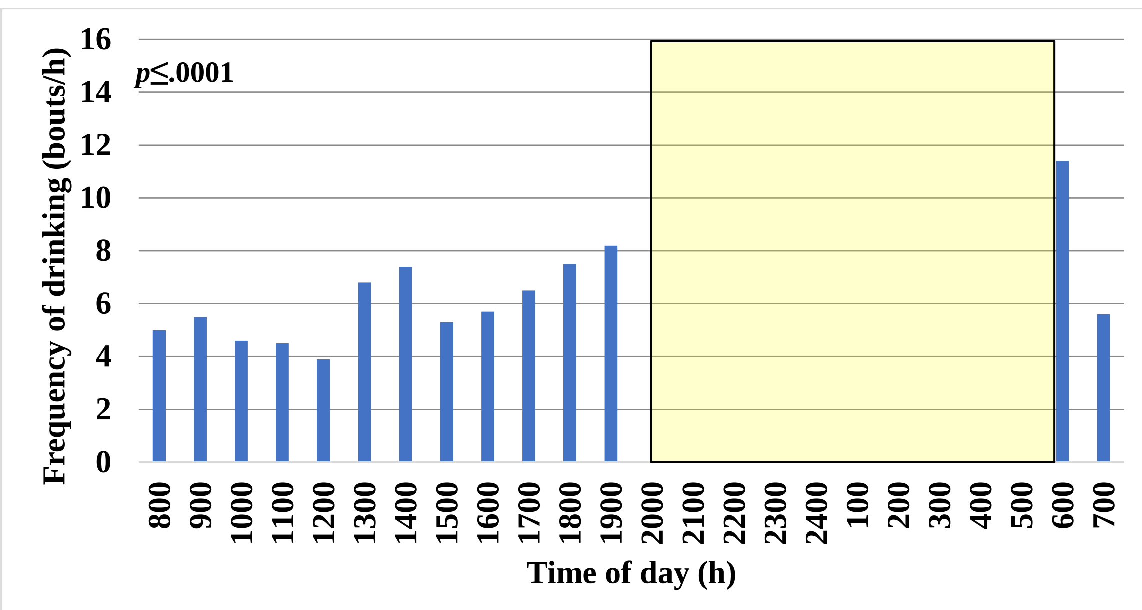

FIGURE B12. Frequency of drinking during the photoperiod and scotoperiod by 2 week old male broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

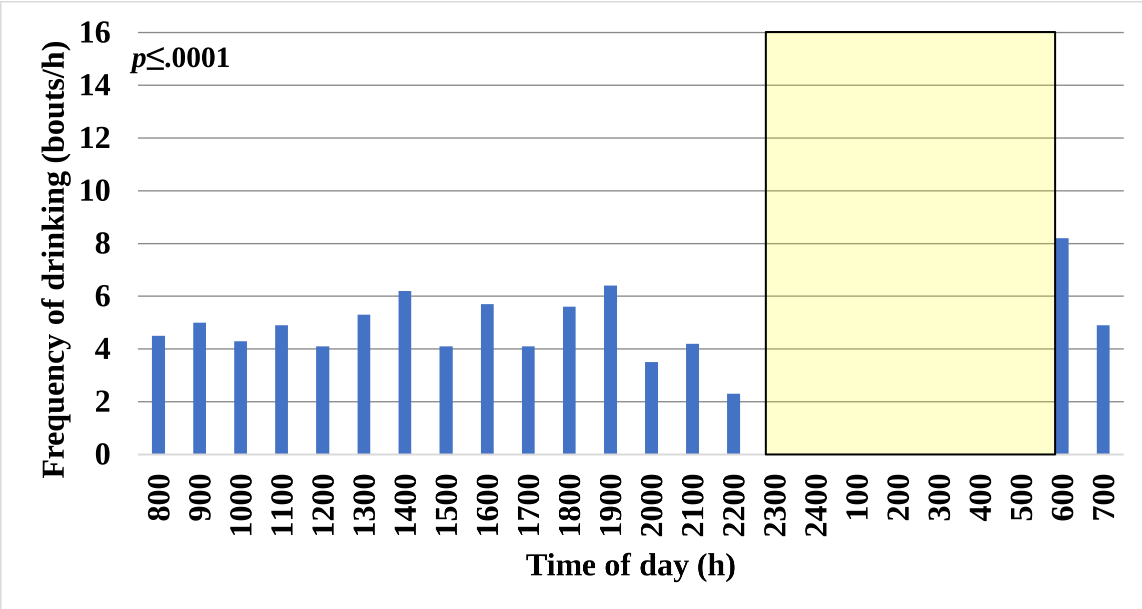

FIGURE B13. Frequency of drinking during the photoperiod and scotoperiod by 2 week old male broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

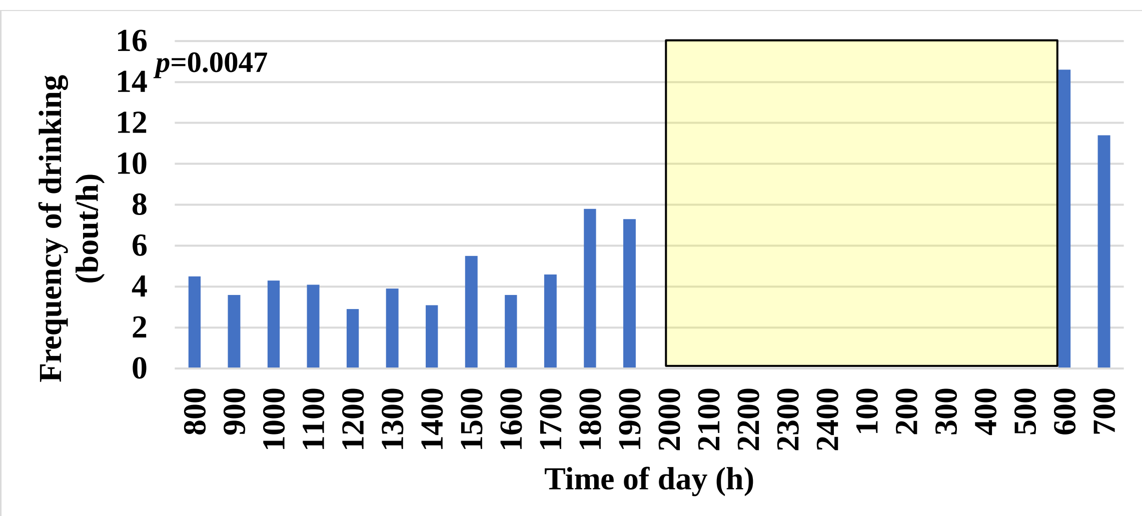

FIGURE B14. Frequency of drinking during the photoperiod and scotoperiod by 4 week old male broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

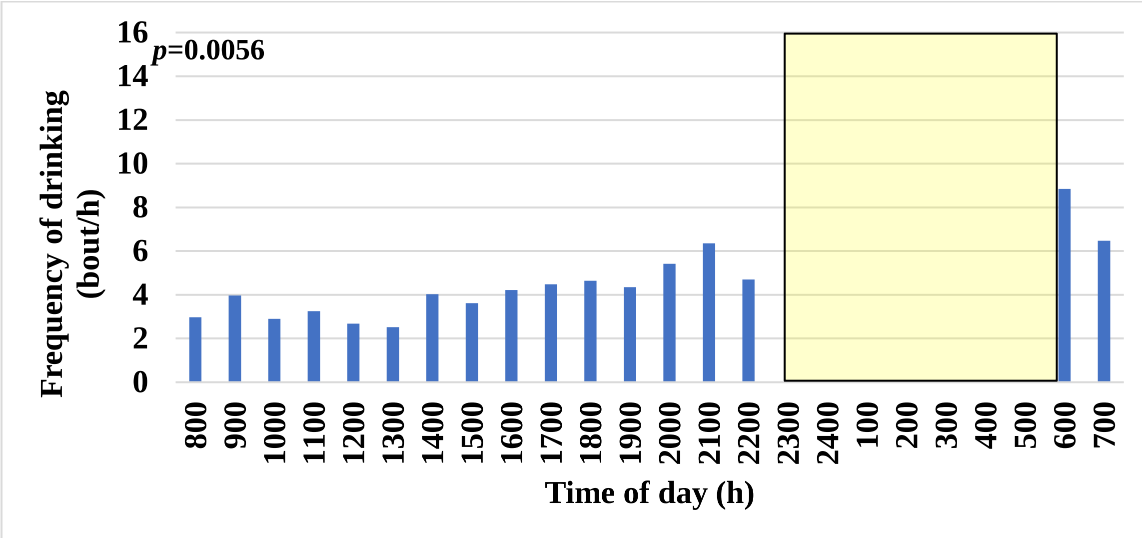

FIGURE B15. Frequency of drinking during the photoperiod and scotoperiod by 4 week old male broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

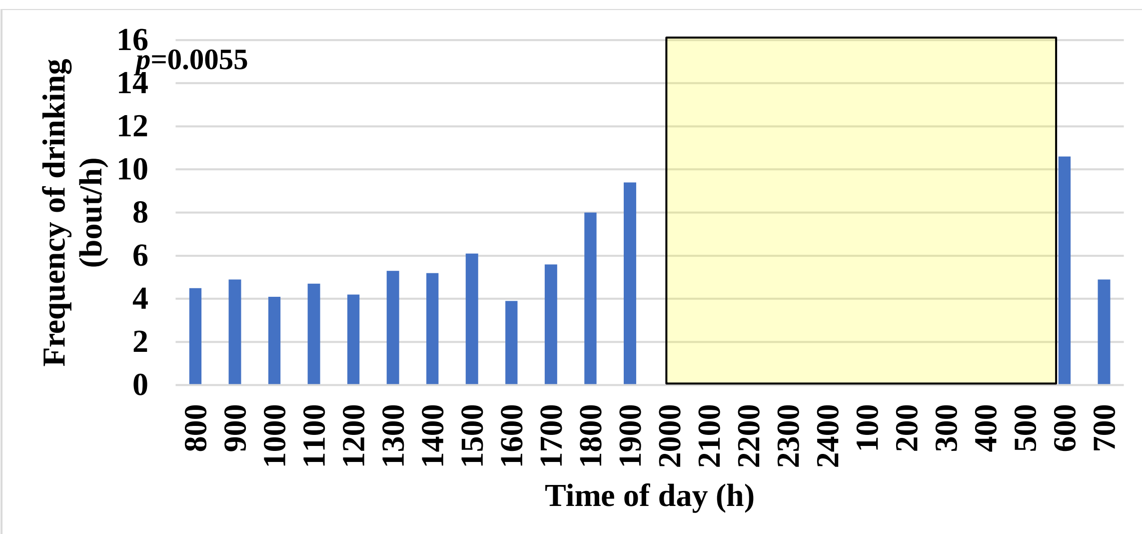

FIGURE B16. Frequency of drinking during the photoperiod and scotoperiod by 2 week old female broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

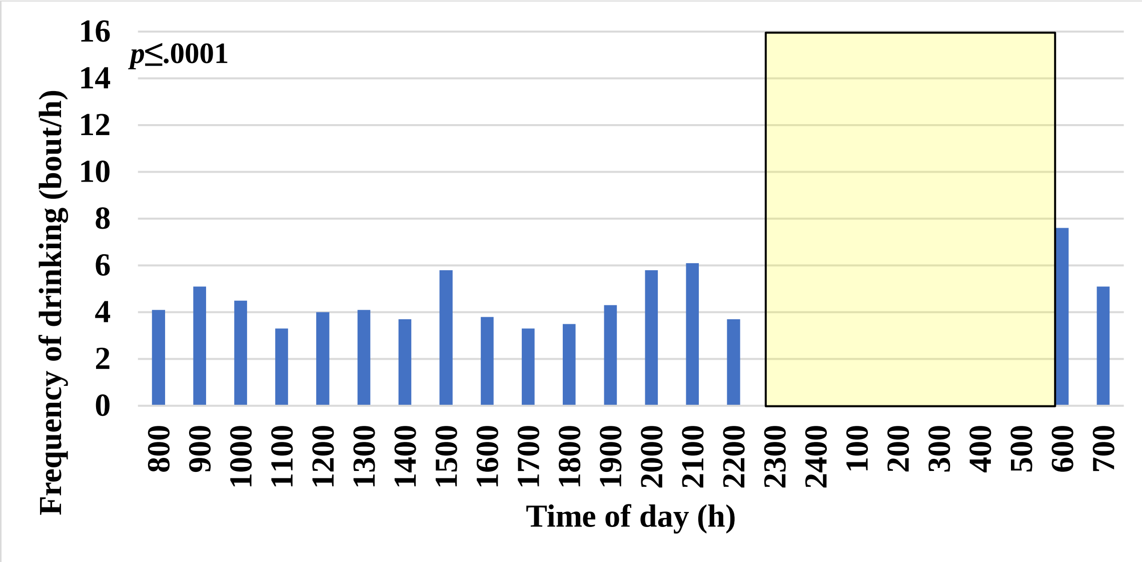

FIGURE B17. Frequency of drinking during the photoperiod and scotoperiod by 2 week old female broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

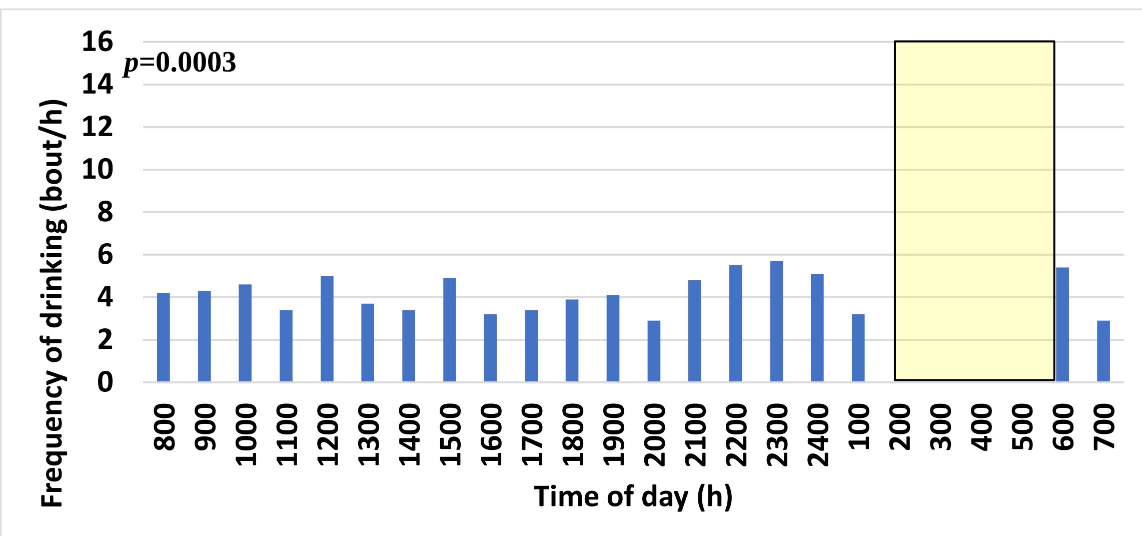

FIGURE B18. Frequency of drinking during the photoperiod and scotoperiod by 2 week old female broilers exposed to 4 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

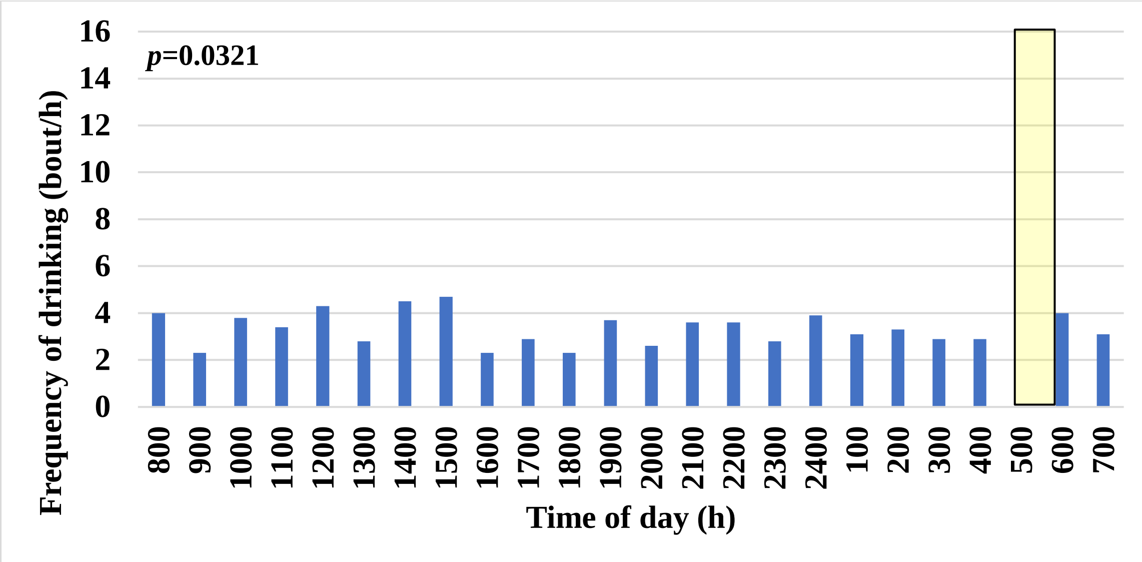

FIGURE B19. Frequency of drinking during the photoperiod and scotoperiod by 2 week old female broilers exposed to 1 hour of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

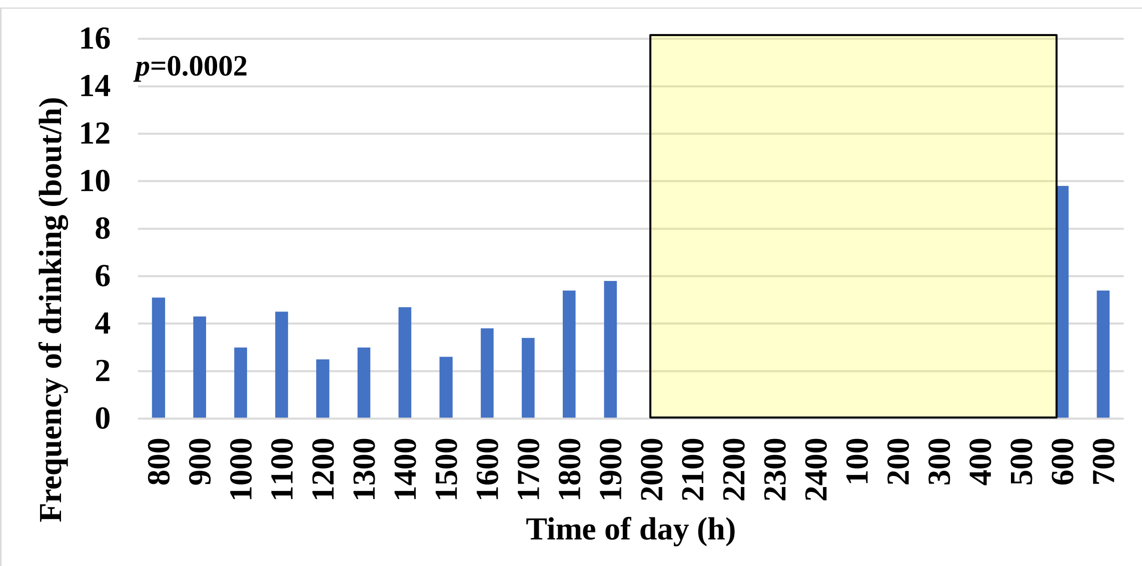

FIGURE B20. Frequency of drinking during the photoperiod and scotoperiod by 4 week old female broilers exposed to 10 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.

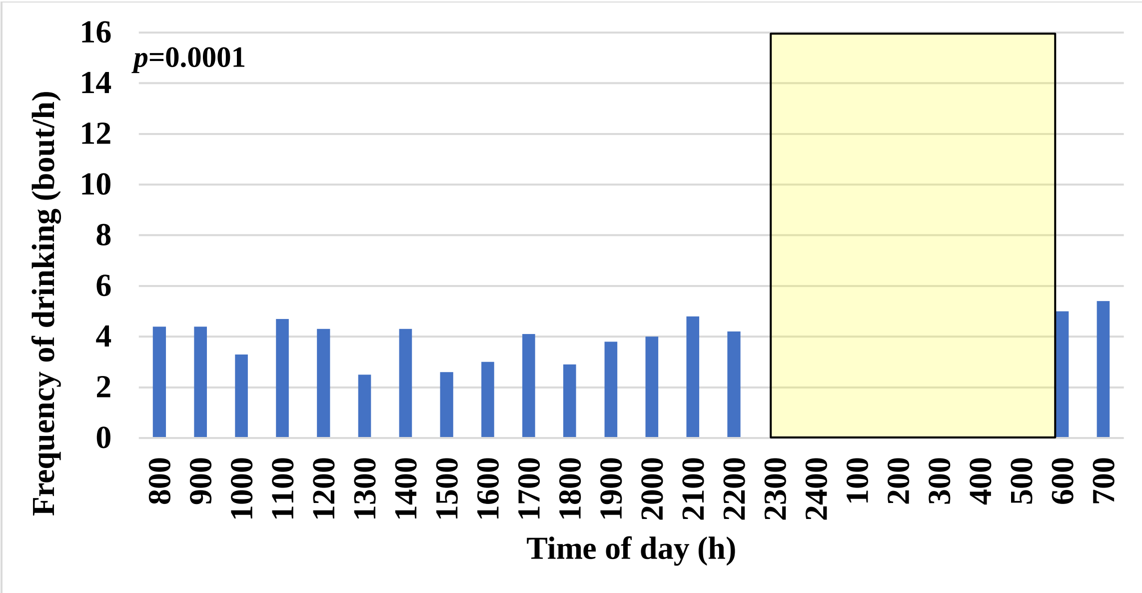

FIGURE B21. Frequency of drinking during the photoperiod and scotoperiod by 4 week old female broilers exposed to 7 hours of darkness. The shaded box represents the scotoperiod. The P value indicates a quadratic response.