

The Importance of Daylength and Darkness Exposure on the Welfare and Productivity of Commercial Broilers

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Submitted by

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**LIFE IS CHANGE
PERSEVERE**

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Overall Abstract

The impact of daylength on commercial broilers was studied in two experiments, with birds exposed to one of four lighting programs (14L:10D (14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L)). The first experiment was repeated in a block formation four times, with each block containing two full-room replications and including a total of 16,128 broilers. In addition to daylength, other main effects studied in this experiment were genotype (Ross x Ross 308 and Ross x Ross 708) and gender, and these variables were randomly placed within each room. The second experiment included two replications of each of the four lighting programs described above in a format similar to a commercial situation. The total number of birds in this experiment was 7,552, equally distributed into one of eight rooms, with each room containing equal numbers of male and female Ross x Ross 308 broilers. Lighting programs used graded levels of daylength, which allowed the use of regression analysis as the primary statistical tool to define relationships between variables and daylength (SAS Proc Reg and Proc RSReg). For data involving light, genotype and gender (productivity, mortality, gait score, ocular weights), statistics included SAS General Linear Model to test for differences in genotype and gender, and interactions between the three main effects. In all cases, data not normally distributed were ($\log+1$) transformed prior to analysis. In experiment 1, daylength and body weight were quadratically related at 32 and 39 d of age, with 20L producing the heaviest birds. Although not statistically significant, the data curve at 49d was of similar shape, with both 17L and 20L birds heavier than those raised under 23L. With less replication in experiment 2, differences only approached significance at 31 d of age ($P=0.09$), but once again, the regression curve was similar in shape. Birds raised under 20L consumed more feed at 39 and 49 d as compared to those under any other lighting program. 23L birds at 39 d ate as much as those raised on 17L, and less than the similar group at 49d. Feed efficiency was impacted quadratically, and birds

given the longest dark period were most efficient. The latter birds exhibited the lowest mortality levels, which increased linearly with daylength. Both metabolic and skeletal mortality levels increased linearly with daylength in all periods, and infectious disorders in a quadratic fashion, with the peak found in birds raised under 20L. Average gait score was reduced either linearly or quadratically with increasing daylength, and footpad health at 28 and 35 d of age decreased as daylength lengthened. Eye weight was heavier under 23L only. The behaviour of 2 pens per lighting program of Ross x Ross 308 males was recorded with the use of infrared cameras within two of the experiment 1 trials (27/28d in trial 1 and 42/43 d in trial 2). Using scan sampling techniques at 10 min intervals, the average percentage of time performing various behaviours was calculated for the photophase, scotophase, and 24 h period (6 measurements x 24 h per d). Standing, walking, feeding preening, stretching, dustbathing and foraging decreased (linearly or quadratically) with increasing daylength at both ages in both the photophase and 24 h period. Running was reduced in a similar manner at 27/28 d, and was completely eliminated under 23L. These latter birds no longer performed dustbathing behaviour at 42/43 d of age. Percent of time of all behaviours was similar or only slightly different for birds raised under 14 and 17L. Reactivity was reduced in birds raised under 23L, was intermediate under 20L, and similar for 14L and 17L at 27d of age. To determine if melatonin within the flock was produced in diurnal rhythms, blood samples were collected either six (23L) or eight times (14, 17 and 20L) over the 24 h period (six per time). Samples were analyzed using radioimmunoassay. Diurnal melatonin production patterns were noted for flocks under 14, 17 and 20L, but were not found in 23L flocks. Frequent significant quadratic or linear relationships were noted between behaviour and time during the photophase under both 14 and 17L, and occasionally under 20L. No such relationships were noted under 23L, indicating sporadic timing of behavioural expression amongst the flock. The lack of flock synchronization under 23L may lead to repeated sleep disruption (fragmentation), which results in similar symptoms to total

sleep deprivation, including poor productivity, a reduction in health, and poor reactivity leading to increased lethargy.

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List of Abbreviations

L	light
D	dark
d	day
h	hour
m	meter
cm	centimeter
kg	kilogram
g	gram
s	second
AME	apparent metabolizable energy
GS	gait score
FPLS	footpad lesion score
ACTH	adrenocorticotropic hormone
SWS	slow wave sleep
FWS	fast wave sleep
REM	rapid eye movement
nREM	non-rapid eye movement
EEG	electroencephalogram
$Y=mx+b$	Y =dependant variable; x =independent variable; m =slope; b =intercept

Chapter 1. Literature Review: The impact of light and dark exposure on welfare and productivity of broilers

1.1. Introduction

The broiler industry across the world can be quite diverse, and management styles can vary from one area to another. The use of lighting programs in management practices is no exception. Daylength for use in commercial broiler systems is regulated in some localities. An official directive published by the Council of the European Union (2007) has regulated the minimum amount of darkness required for broilers. It states that the inclusion of darkness must be initiated by 7 days (d) of age, and must remain in place until at least 3 d prior to marketing. A minimum of 6 hours (h) must be included in every 24-h period, and the program must include one period of at least 4 h of continuous darkness. Other areas of the world, including Canada, have minimal or no regulation for daylength requirements. The Animal Care Program of the Chicken Farmers of Canada allows continuous light to 5 d of age, and then requires only 1 h of darkness past that point (Chicken Farmers of Canada, 2009). This decision by the Canadian group is likely based on the belief that a long daylength improves broiler production and the ease of catching birds prior to market. It is not based on bird welfare. Interestingly, the 1 h of darkness is not provided as a period of rest or sleep, but to allow the birds to become accustomed to darkness if by chance a power failure should occur (Savory and Duncan, 1982). Preference testing of broilers in which they are allowed to choose the amount of darkness per day indicates that their preference is for approximately 4 h per day (Savory and Duncan, 1982).

To understand how management practices in poultry production will impact bird welfare, it is important to have an understanding on what animal welfare means. There are many definitions of welfare, and they can vary from person to person. One of the most repeated definitions was put forth by Broom (1996), who explained that welfare is determined by how an animal can cope in its environment. This is an all-encompassing definition, as an animal can cope with changes or stressors in its environment through a number of mechanisms, either

physiological (changes to heart rate or respiration, initiation of the hypothalamus pituitary axis, etc.) or behavioural (loss of behaviours that occur when an animal is comfortable in its environment, flight movement away from the stressor, etc.).

When developing an environment for housing livestock, a set of minimum standards can help to determine what is necessary for the animal to maximize its well-being. Such a set of standards was developed by the Farm Animal Welfare Council (FAWC, 2011). The “5 Freedoms” have been rewritten from their original form, and now contain more descriptive categories. They are comprehensive, and cover biological, physiological and behavioural parameters that must be met for welfare to be maximized. The Freedoms are:

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

The impact that exposure to darkness has on broilers has been studied for decades. Lack of darkness in a broiler photoperiod program has been shown to have a number of negative implications based on biological or physiological processes. Alterations to daylength have been used as a simple management tool primarily for the control of mortality, particularly due to skeletal and metabolic disorders (Classen and Riddell, 1989; Lewis et al., 1996; Brickett

et al., 2007b). In many of these research programs, the studies have compared one lighting program to another, rather than assessing the relationship between daylength and the variable being studied. Often the results have focused on productivity, and the results have been variable. In terms of welfare, many of the projects have focused on health alone. Few research programs have examined broiler behaviour under different lighting programs, and the majority has only studied behaviour during a portion of the day period. Finally, there have been suggestions by some that long photoperiods may disrupt circadian patterns including sleep in broilers. This literature review will document major works previously conducted in these research areas.

1.2. Vision in birds

Vision is likely the most important sensory tool available to domestic poultry (Prescott et al., 2003). Light, therefore, is essential, and all aspects of light have been shown to have an important impact on their behaviour and physiology (Lewis and Morris, 1998). Light is the primary zeitgeber in the control of biological rhythms (Marrus et al., 1996; Middleton et al., 1996; Rattenborg et al., 2005; Kristensen et al., 2006), and to be able to do this, it must be perceived by the brain. The mechanism of action in the bird is different than for most species, as light perception can occur via the retina as it does in other species or, at appropriate light intensities, can penetrate through the skull to directly affect photoreceptors in the pineal gland and the hypothalamus (Prescott et al., 2003). The pineal gland also serves as a circadian clock in the body (Takahashi and Menaker, 1984) while the hypothalamus serves a vital role in initiating the sequence of events required for reproduction. This starts with the release of gonadotrophin releasing hormone, which in turn affects luteinizing hormone and follicle stimulating hormone produced in the pituitary gland (Lewis and Morris, 2006). When light comes in contact with

photoreceptors in the retina and pineal gland, photons are transformed to neurological activity which passes this signal to the hypothalamus.

The shape of the eye is important for the vision of birds. Chicken eyes are large and relatively flat (Lewis and Morris, 2006). This is important, as the shape allows the bird to adjust the refraction capability (Prescott et al., 2003), which in turn allows birds to see near and far objects. This adjustment of refraction capabilities is called accommodation. By adjusting the accommodation within the eye, the bird can focus with the lower section of the eye on nearby objects, and on far-off objects with the upper section (Prescott et al., 2003). This is useful in many situations, such as foraging and predator observation (Lewis et al., 2006).

1.3. Impact of darkness on productivity

Darkness can be provided in a broiler photoperiod program in a number of ways. These can include darkness being given in one section per day with a consistent length throughout the production cycle, one section per day with a changing amount of light and dark as the production cycle progresses (increasing or decreasing photoperiod programs), with a particular amount of darkness per day split into sections (sectioned), or with repeated light and dark cycles over a 24-h period (intermittent). While the impact of each of these types of programs may vary slightly with respect to their impact on productivity and welfare, in general terms darkness exposure has many similar benefits regardless of the delivery method. Of these types of programs, the majority of research studied has tended to focus on intermittent programs or increasing/decreasing programs when compared to constant or near-constant light. Programs studying only the impact of varying daylength alone have been limited.

1.3.1. Growth and feed intake

Within the current published literature, there is conflicting evidence as to the impact of daylength on the growth of broilers. Traditional theory indicates that the use of constant or near-constant light results in maximum productivity, as birds are in an environment with unlimited visual access to feeders and waterers. This philosophy is still apparent with many commercial producers today. The evidence to support this theory stems from as early as the 1950s. Moore (1957) reported in abstract form that constant or near-constant photoperiods resulted in both maximum growth rates and feed intakes when compared to daylengths of 6, 12 or 18 h. Only small numbers of birds were used (20 broilers per sex for each treatment) and only three replicates per treatment. The data were further confounded by darkness in the 6, 12 or 18 h programs being provided in one or even more sections per day. It is therefore interesting that this abstract is often used to substantiate constant light resulting in the best growth rate. Sørensen et al. (1999) reported four experiments to test daylength, with two lighting programs per experiment. The daylengths tested were 8, 16, 21 and 23L per day. The results showed heavier weights on the long daylengths, but it is important to note that all four daylengths were not compared in one experiment. Therefore, it is not appropriate to make a conclusion based on the range of all four daylengths. This, combined with restricted feeder space in this work, again draws into question the conclusion that long daylengths result in maximum body weight. Other research has similarly found that equal or heavier weights for broilers are achieved under continuous light. A very recent comparison between broilers on 16L and those on 24L raised to 42 d showed no differences in body weight or in feed conversion ratio (FCR) (Bayram and Özkan, 2010). Only four pens within one room per lighting program were used (total number of birds = 184), so had there been an effect of room the data could be skewed. In many research projects, continuous light has only been compared to one other lighting program, so a response curve could not be established. For example, Savory (1976) compared broilers raised on 23L to

those on 12L to 10 weeks (wk) of age, and found those on 23L were heavier. Birds were also heavier when exposed to 23L per day as opposed to those on 12 h of light when grown to 42 d of age (Ingram et al., 2000). No daylengths between the very restrictive 12 h and the long 23 h were included in either of these experiments, which again demonstrate that constant light results in superior body weight. One research program did compare a range of photoperiod lengths ranging from 2 to 24 h (2, 4, 6, 8, 10, 12, 15, 18, 21 and 24). Both Ross 308 and Cobb 500 broilers were compared in this work (Lewis et al., 2009a). No differences were noted in growth rate or feed intake to 35 d within the range of photoperiods from 6 h through 24 h. Two experiments were conducted, but within each only one pen per genotype in one room was used for production for each of the lighting programs. Between experiments, bird density differed, and one less lighting treatment was included in the second; thus, combining experiments for statistical analysis may not be appropriate. Hence, no photoperiod replication was involved in each experiment. To the author's knowledge, no one has compared a range of daylengths to constant or near-constant lighting programs with appropriate replication. Therefore, it may not be possible based on existing literature to establish that constant light results in heavier birds.

One of the suggestions for why birds on short photophase programs might be lighter in weight is that a long dark period restricts feed intake by reducing available feeding time (Classen, 1992). Birds do preferentially feed during the light period (Classen and Riddell, 1989), but this pattern can be adjusted in broilers exposed to longer dark periods. Lewis et al. (2009b) compared broilers on 8 h of light per day to those on 16 h, and saw an increase in night time feeding in the 8 h birds to the point where approximately half of their daily feed intake occurred during the dark. At 35 d, no differences were noted in body weights between the two groups, indicating that broilers given 8 h of light were able to adjust to the long period of darkness. In a separate research project, Lewis et al. (2009c) found night time feeding to occur in birds given 15 h of light or less per day, starting at 5 d of age, based on feeder weights at the

end of day and start of following day. Factors that might affect this could include allowable feeder space, diet density, or perhaps light infiltration into the rooms. No mention of the light-tightness of the rooms was given.

A reduction in broiler growth rate immediately after the introduction of a dark period is well established in research, but the long-term effects on growth and body weight are less well defined. It can be hypothesized that the longer the time between introduction of darkness and measuring body weight, the less impact there would be on body weight. This hypothesis appears to hold true in the literature. Charles et al. (1992) found that broilers given a lighting program where daylength increases over the grow-out period (increasing lighting) weighed less at 5 wk of age than those exposed to continuous light. However, at 7 wk, the birds given increasing lighting were heavier than the continuous light broilers. It should be noted that, in this case, increasing lighting program birds were at near-continuous illumination at market age, and this could have an impact. As well, testosterone may be higher because of the changing and increasing lighting program, which again could impact growth rate. Riddell and Classen (1992) found similar data with increasing programs. Broilers on constant light were heavier at 42 d, but lighter at 63 d. No differences were noted in feed intake or feed efficiency. Renden et al. (1993) reported lower body weights for birds on 16L or an increasing program as compared to near-constant light birds at 42 d; but by 48 d, the differences had disappeared. Buyse et al. (1994) compared near-continuous programs to either step-up or step-down lighting programs, and found growth on the near-constant program to be equal or poorer than that achieved with the changing programs. They emphasized that introducing darkness at 7 d of age reduced early growth rate, but that compensatory gain had taken place later in the grow-out cycle to allow body weight to be at least as good as that achieved on the near-constant program. Similarly, Rozenboim et al. (1999) found that birds raised to 42 d under 23L:1D were heavier than those exposed to either an intermittent or increasing program with the inclusion of 8 h of

darkness; however, by 49 d these differences had disappeared, again demonstrating slower initial growth and faster growth later in life when birds are exposed to darkness. This faster growth curve later in life has been termed compensatory gain, and the slower initial growth and faster compensatory gain later in the grow-out period appears to be a shift in the growth cycle of birds exposed to darkness (Classen and Riddell, 1989; Buyse et al., 1996a, b). This compensatory gain has been attributed to an increase in growth hormone production (Buyse et al., 1996a, b), which in turn can be influenced by melatonin secretion (Zeman et al., 1999).

How much darkness is given to broilers can also impact growth. Classen (2004) compared birds exposed to 12, 16 or 20 h of light per day and found a linear relationship between growth and daylength, with the longest light periods having the highest growth rates. No comparison with continuous or near-continuous light was made, so it is not possible to determine if the linearity of the growth pattern would occur beyond 20 h of light. Brickett et al. (2007a) found that adding 12 h of darkness reduced market body weight at 35 d as compared to a 20L photoperiod program. Again, insufficient daylength treatments preclude establishment of growth response over a range of daylengths. Based on the research mentioned above, very short daylengths such as 12 h may require longer periods of time, or older ages of birds, to allow the adjustment to darkness to occur and to allow time for compensatory gain to take place. This suggests that the choice of dark period inclusion for productivity of birds should partially be based on the age at market.

1.3.2. Feed efficiency

Daylength has been reported to affect feed efficiency (FCR), and the majority of research supports the concept that shorter daylength (longer dark period) improves FCR. In a comparison of two constant photoperiods (8 h or 16 h) and an increasing program (birds exposed to a changing photoperiod ranging from 8 to 16 h of light per day by 21 d of age)

(Lewis and Gous, 2007), the 8 h birds and the increasing program birds were more feed efficient than the 16 h birds. Brickett et al. (2007a) found that birds exposed to 12 h of darkness were more feed efficient than birds exposed to 4 h of darkness. Similarly, birds exposed to 23L vs. 12L resulted in heavier weights for the 23L birds at 42 d, although feed conversion was poorer under the long daylength photoperiod (Ingram et al., 2000). A similar experiment exposed broilers to a 14L day, and at 49 d of age these birds were equal in body weight to the 23L birds; however, the 23L exposed birds had poorer feed efficiency (Kühn et al., 1996).

The pattern of darkness exposure also plays a role in FCR, with a single longer dark period being superior to the same length of daily darkness broken down into two or more periods. For example, Classen et al. (2004) found better FCR in birds exposed to 12 h scotoperiods than those exposed to two 6-h periods or twelve 1-h periods.

There are likely a number of contributing factors to darkness improving FCR. Providing longer dark periods increases the time period when birds have lower metabolic rates, thereby reducing energy requirements (MacLeod et al., 1980; Boon et al., 2000a; Classen et al., 2004). As well, melatonin, a neurotransmitter produced in both the retina and the pineal gland of the chicken and which is involved in the regulation of many circadian rhythms in the body, peaks during the dark period (Hau and Gwinner, 1994). Exogenous melatonin has been shown to result in the onset of sleep (Hishikawa et al., 1969; Bermudez et al., 1983), and other evidence has shown that the addition of exogenous melatonin to the diet of broilers results in reduced feed intake and an improvement in feed efficiency (Clark and Classen, 1995; Appeldoorn et al., 1999). Why this occurs has not been established, but it could be related to a reduction in energy expenditure (Appeldoorn et al., 1999) that might be associated with altered endocrine status (T_3 or T_4) or an enhanced glucose uptake in the jejunum with increased melatonin (Osei et al., 1989). Finally, activity also declines during the dark period, reducing energy requirements

further (Classen, 2004). For example, MacLeod et al. (1980) found exposing breeding and growing birds to 1 h darkness per day reduced energy expenditure by 12% over the photophase section of the day, but exposing them to a 10-h period decreased the energy expenditure by 22%. A similar energy conservation pattern has been found in Japanese quail exposed to darkness periods of 6 h to 18 h (Boon et al., 2000a).

1.3.3. Health

There are many factors that can be involved in the determination of welfare assessments in agricultural animals, but the most obvious indicator is health. Constant light is thought to increase the incidence of mortality and morbidity in a broiler flock, primarily by impacting levels of leg disorders, metabolic disorders, and possibly immune function (Lewis and Perry, 1986; Classen and Riddell, 1989; Brickett et al., 2007b).

The data in the literature, however, are variable with regards to darkness and its impact on mortality levels. Lewis and Gous (2007) compared two constant programs of either 8 or 16 h to an increasing program (ranged from 8 to 16 h at 21 d of age), and found no impact on mortality. Ingram et al. (2000) compared birds on 1 h of darkness per day vs 12 h, and found no mortality differences. In contrast, Brickett et al. (2007a) found lower mortality when birds were exposed to 12 h darkness per day vs. 4 h. There could be multiple reasons for this, including the daylengths compared in the research. For example, it is possible that 8 h of darkness is enough to result in low mortality and that increased darkness exposure (16 h) has no or little additional benefit. Statistical significance is also very difficult to achieve with mortality and cull levels. Contributing factors to this include a relatively low incidence, high variability amongst replicate units and, in some cases, relatively small numbers of birds (Riddell and Classen, 1992).

1.3.3.1. Skeletal health

Leg weakness is often cited as one of the main concerns for broiler welfare, as pain can be an issue (Blokhuys, 1984; Danbury et al., 2000; McGeown et al., 1999; Sanotra et al., 2003). The term “leg weakness” is broad, and includes anything that causes lameness in birds (Nairn and Watson, 1972). Hence, it includes both infectious and non-infectious disorders. Reducing leg weakness has been one of the primary purposes for using darkness in a lighting program for many years, and the majority of research comparing continuous or near-continuous light to programs with darkness exposure has shown that darkness exposure can improve leg health. Regardless of the type of darkness exposure used, there is evidence in the literature to substantiate this. Examples include Robbins et al. (1984), who compared birds on constant light versus those exposed to 18L:6D, and Classen and Riddell (1989) and Classen et al. (1991) whose work used an increasing lighting program, in which birds are exposed to short daylengths that gradually increased over time.

Levels of skeletal disease can vary immensely in the literature. For example, Kestin et al. (1992) reported that 90% of birds show some abnormality of gait, and that 26% of those represent a welfare concern. Comparing that to other literature published at approximately the same time, Renden et al. (1991) found leg abnormalities to occur in 22% of a flock raised on constant light and 16% in a flock raised on an increasing lighting program. Sørensen et al. (2000) reported numbers to be relatively low – less than 1% falling in the upper GS categories of 4 and 5. These numbers vary considerably, but many factors could play a part. Primary breeding companies appear to have used different selection pressure and selection techniques to reduce the incidence of leg weakness; this is demonstrated by strain variation in skeletal disease levels (Kestin et al., 1999). Age of leg weakness assessment is also a factor, as the incidence of leg weakness increases with age. How leg weakness is measured also plays a role; this is particularly true for gait scoring, which is a subjective measurement.

Leg weakness represents an economic loss for producers, as a result of death loss and culling as well as a reduction in performance, because birds are likely not eating and drinking at the same frequency. The latter point is indicated by behavioural observations in some research. For example, Weeks and Kestin (1997) found that lame birds spent only half as much time standing as did healthy birds. More importantly, leg weakness represents a reduction in bird welfare. Lame birds are often light in body weight (Yalçin et al., 1998; Julian, 2005), indicating that getting to the feeders and waterers is an issue (Sørensen et al., 1999). This again indicates a welfare issue, as broilers have a large motivation to feed (Bokkers et al., 2004); restricting a highly motivated behaviour is indicative of a reduction in welfare.

The etiology of leg weakness appears to have changed over time. The most common diseases reported in the early 1980s were twisting or bowing of the intertarsal joint (valgus or varus deformities) and tibial dyschondroplasia (TD) (Randall and Mills, 1981). A report from 1994 indicated the highest leg weakness incidence was long bone distortions or valgus/varus deformities, and that these deformities did not exhibit a reduction in calcification in the growth plate that had previously been seen with TD-inflicted birds (Hester, 1994). The change in TD levels noted over this time frame confirm a change in causative factors of leg weakness, either brought on by selection pressure by primary breeding companies or through natural changes over time. It also pointed out that leg weakness still was an issue, although the causes may have changed. Kestin et al. (1992) pointed out that the primary cause of leg weakness was likely non-infectious, and Julian (1998) and Sanotra et al. (2001) found the primary diseases involved were TD lesions followed by angular deformities. Berg and Sanotra (2003) found that moderate to severe leg weakness issues were present at a rate of 13.5% but that, on the whole, 20.8% of the flocks surveyed had angular limb deformities.

There are other factors that impact levels of leg weakness. Males have close to twice as many defects as do females (Randall and Mills, 1981). The change in muscle composition of birds has also appeared to impact the mobility of birds, and those with larger breast muscle development have an altered gait. This appears to result in a very inefficient walking type, which has been suggested to tire birds more rapidly (Corr et al., 2003). Mench (2004) agreed that confirmation could impact mobility, but suggested that birds falling in the extreme high gait scores were not doing so because of conformational differences but because of skeletal deformities.

Rapid growth rate is often used to explain incidences of leg weakness. Slowing the initial growth curve and allowing compensatory gain to occur later in life has been shown to reduce skeletal disease; this has been accomplished with both the use of lighting programs containing a dark period (Classen and Riddell, 1990) and by restricting feed intake early in life (Riddell et al., 1983; Robinson et al., 1992). Feed restriction has not been adapted by industry as a method of reducing the incidence of leg weakness, but lighting programs are commonly used.

Slowing the growth rate early in life to reduce leg weakness with feed restriction has not been successful. A study by Leterrier et al. (1998) slowed the growth rate of broilers by feeding low energy feeds (28% reduction in energy). At 12, 26 and 42 d of age, the tibia bone characteristics were different between the two bird treatments. Bone weight, volume (as measured by water replacement), length, cortical area around the mid-diaphysis, structure (active areas of osteons within the bone) and bone ash were reduced in the slower growing birds, but by 46 d no differences were noted. Other characteristics did not differ, such as dry defatted weight and percentage of ash as compared to defatted weight. When the birds were compared at equal body weights, bone characteristics were similar between the two groups of

birds. The incidence of valgus-varus deformities, however, was significantly lower in the slow growing birds. The conclusions from this work suggest that growth rate when slowed by dietary means does not result in an improvement in cortical bone quality.

The inclusion of darkness in a photoperiod program has been shown to reduce leg disorders in broilers (Classen and Riddell, 1989; Renden et al., 1991; Riddell and Classen, 1992; Manser, 1996) and improve the walking ability within the remaining flock (Sanotra et al., 2002). Valgus and varus deformities, which are an inward or outward curvature of the leg, can be reduced with the use of darkness (Leterrier and Constantin, 1996). There have been a number of mechanisms implicated for this improvement.

Exercise reduces skeletal disease in many species. For example, basic standing exercises are now being used for immobilized humans to stop the decline in bone density (Goktepe et al., 2008). Exercise is also commonly implicated as a factor affecting leg weakness in broilers (Wilson et al., 1984; Reiter and Bessei, 2009). Evidence from both avian and mammalian species strongly supports the concept that a lack of exercise (inactivity) reduces skeletal quality. Broilers must be active to some degree within a large facility to be able to eat and drink. How much exercise is required to establish normal bone growth and development is less well defined, so the impact on broilers may vary. Hence, increasing exercise levels may be part of the reason for better leg health when photoperiod programs with the inclusion of darkness are used (Classen et al., 1994; Balog et al., 1997; Reiter and Bessei, 2009).

There is evidence of a positive relationship between exercise and leg health in the literature. Reiter and Bessei (1996) compared groups of birds for which the distance between feeders and waterers was increased, and found a reduction in the angular and torsional deformities of both the tibia and femur bones. The same group (Reiter and Bessei, 2009) compared the ability of slow and fast growing broilers to walk on treadmills. Throughout the

grow-out period, slow growing birds walked significantly farther per hour than the fast growing birds. The slow growing birds exhibited better bone density and bone thickness as well as a reduced level of twisted bones. Based on these results, Reiter and Bessei then compared commercial flocks with feeders and waterers within 2 m of each other or farther apart (12 m apart), and found that the latter group walked farther, had less leg weakness issues in the flock, and reduced mortality attributed to leg disease, without a negative impact on body weight or feed efficiency. However, a similar study that varied distances between feeders and waterers for groups of turkeys did not produce the same results (Noble et al., 1996). In this work, activity was increased with larger distances, but no improvement in bird mobility was noted. Sherlock et al. (2010) used varying light intensities to attempt to influence activity; in their work, brighter intensities had an effect. However, the change in exercise levels did not impact skeletal health. Bizeray et al. (2002) found that attempting to increase exercise levels by creating a more complex environment for broilers to live in did not improve bone quality or breaking strength. This conflicting evidence could suggest that how much exercise is necessary to reduce leg weakness issues in broilers and in turkeys has not been quantified. It could also be that mobility in these flocks (control and treatment) was generally good, and that exercise may be more important when leg weakness issues are more severe.

In chickens, a number of different lighting programs have been used that result in a higher activity level and concurrently reduced bone disease. Hester et al. (1983) used step-up vs. step-down lighting for turkeys. Results showed that leg weakness was reduced and activity was higher for the step-up program birds. However, this lighting program also appeared to speed sexual maturation, which could impact leg health. A similar experiment with broilers noted less leg defects in broilers exposed to 6 h light to 21 d then 23 h L and an increasing program in one of three experiments than a consistent 23 h L (Classen and Riddell, 1989). Although only a subjective note, behaviour appeared to be more active in the groups with

darkness exposure. Again, however, sexual maturation appeared more advanced in both the 6-23 h L group, and followed by the increasing program groups, which further complicates the discussion of exercise and leg health. Sanotra et al. (2002) reported increased activity, as measured by behavioural observations for a period of the photophase, and reduced leg weakness in broilers exposed to 8 h D compared to birds given constant light. Ferrante et al. (2006) compared broilers exposed to 8 h darkness per day to those given 23L:1D per day, and found both an increase in activity and a reduction in foot pad lesions, which can lead to reduced mobility, under near-constant light. While exercise likely does play an important part in skeletal health, these studies did not separate the impact that exposure to darkness provided from the exercise component. Thus, exercise is likely not the only variable impacting bone health and skeletal disease, and other factors may be important as well.

Although not always the case, the majority of research has demonstrated that exposure of broilers and turkeys to darkness has a positive effect on leg weakness. However, this conclusion does not lead to establishing the mechanism(s) whereby darkness has its beneficial effect. Darkness results in a variety of consequences that may be involved, and it is difficult to separate these effects. As a consequence, the interpretation of many lighting studies on this topic is confounded.

Classen and Riddell (1989) suggested that important metabolic changes take place in response to darkness exposure itself, and these may be involved in bone remodeling. This would act regardless of growth curves and might explain why darkness impacts bone mineralization; however, slowing growth rate through diet dilution does not always accomplish the same thing (Leterrier et al., 1998).

Providing darkness early in life shifts the growth curve, as explained in the previous section on light and productivity. By slowing early growth rate, it is thought that the skeletal system is then allowed to form a strong foundation prior to heavy weight being placed upon it (Sanotra et al., 2002). Robinson et al. (1992) used a similar degree of feed restriction during either wk 2, 3 or 4 of broiler life. When restriction was used during wk 2 to adjust this early growth curve, a reduction in leg disorders was noted. Similar data were shown by Su et al. (1999) using early feed restriction. However, this is not the only mechanism involved. Classen and Riddell (1990) matched growth rate of birds on a near-constant photoperiod to those with darkness inclusion using feed restriction early in life, then monitored health status. Even though body weights were the same, birds with darkness exposure experienced a lower level of skeletal and leg disorders than did those on near-constant light, suggesting that a metabolic change in the birds from darkness itself played a role. This could come from a number of factors. In a comparison of birds on constant light vs. intermittent light, where the birds on the latter program demonstrated slow growth initially followed by more rapid growth later in life, birds on the intermittent program secreted a higher level of growth hormone than did the control birds (Buyse et al., 1997). It is known that light patterns impact melatonin rhythms (Fleissner and Fleissner, 2002) and that melatonin has an impact on growth hormone (GH) production. GH is thought to be the primary hormone responsible for growth (Spencer, 1985). This could possibly be one of the mechanisms involved in controlling compensatory growth. It is also suggested that other hormones may be impacted by the circadian rhythm of melatonin production, which could be involved in the health status of broilers.

Human research has recently pointed to the impact that melatonin production can have on bone health. Sánchez-Barceló et al. (2010) hypothesized that melatonin has three potential mechanisms, although the mechanism for how these work is not yet understood. Melatonin could increase the functioning of the bone-building osteoblastic cells. It may also increase the

osteoblastic expression of the osteoprotegerin, which then functions to inhibit the production of osteoclasts. Finally, it may change the mechanism by which bone is remodeled. If melatonin acts similarly in broilers, then exposure to darkness is a requirement for bone health.

Darkness may not impact all leg weakness diseases similarly. TD is a leg weakness in which an abnormal cartilage plug is found in the growth plate of fast growing chickens and, if severe, results in immobility (Rennie et al., 1995). Riddell et al. (1983) found that restricting feed to slow growth of broilers lowered the incidence of TD, but Wong-Valle et al. (1993) and Garner et al. (2002) found no differences when comparing near-constant light and intermittent lighting.

1.3.3.1.1. Bone quality

Levels of ash in bone are a valid determinant of bone density (Siegel et al., 1973; Thorp and Waddington, 1997), and bone quality may be a reason for improved skeletal health seen with darkness exposure. Bone strength is positively correlated with ash level (Lewis et al., 2009c). As with many other diseases, rapid growth rate has been implicated in poor bone density, and in the tibia, cortices are more porous in faster growing birds (Letierrier and Nys, 1992) resulting in a higher level of leg weakness in a broiler flock.

Photoperiod has been shown to impact bone strength and bone ash through a number of mechanisms, including increases in physical activity with darkness exposure, changes to feeding behaviour, feed intake, and body weight, and changes in hormonal release that accompany day-night cycles (Lewis et al., 2009c). This diurnal pattern of light and dark exposure likely plays a major role in the formation of the bone matrix (Simmons et al., 1979). In rats, the first 4 h of darkness is when the majority of this metabolic activity takes place (Simmons et al., 1979). In birds, the first week of life also seems to be a trigger point for bone development (Lilburn, 1994). This is also true in the human population; Chad et al. (1999)

found early childhood to be particularly important in bone density development and that load-bearing exercises, even for short periods, can be beneficial to bone quality. This factor leads to a question regarding why industry often chooses to expose chicks to continuous light illumination for at least a portion of this period.

Exposure to diurnal light and dark cycles has been shown to affect bone ash or bone quality. Classen et al. (1994) found this in turkeys exposed to either constant, increasing or decreasing photoperiods, where bones from birds on the constant program were weaker. Brickett et al. (2007b) found that increasing daylength to 20L vs. 12L at 4 d of age resulted in a decline in bone ash at 35 d of age, suggesting poorer quality of bone. Contradictory to this data though, are a number of research reports. Ingram et al. (2000) did not find an improvement in tibia breaking strength when they compared birds on 23L to those on 12L at 42 d of age. Lewis et al. (2009c) actually found a positive relationship forming between increased daylength and bone ash when birds were exposed to a number of photoperiod lengths ranging from 2 to 24 h, and has suggested that these differences could arise from the variability in the data or the fact that lighting programs were initiated at a very young age (2 d). Differences in methodology could be a factor as well. Brickett et al.'s (2007b) work used ether extract to defat the bones, but this process was not performed in Lewis's work. Meat yield data from Brickett's work found heavier breast skin weights, which could be indicative of fat content, in birds on longer photoperiods, although this response was only measured in birds raised on the two lighting programs. However, it does raise the issue of variability in bone weights. Because Lewis's work did not defat bones, initial weight of the bones between the two sets of work could be different.

Lewis et al. (2009c) found an interesting response with daylength and bone breaking strength. Two strains were measured in this work. In Ross birds, breaking strength increased when daylength rose from 2 to 7 h, then decreased with longer photoperiods. Cobb birds

followed a similar curve, but the peak occurred under 12 h of light. Perhaps this is a reflection of selection procedures applied by the primary breeding companies. In general, however, tibia breaking strength was negatively correlated with daylength. If photoperiod effects were removed, then breaking strength was actually found to be positively correlated with body weight. This suggests the importance of darkness exposure for bone health.

1.3.3.1.2. Gait score and mobility

Gait score (GS) is a technique used to quantify mobility within a flock of birds. There are two scoring systems that have become standard across the world – Kestin et al. (1992) and Garner et al. (2002). The two systems are similar but have slightly different definitions. However, interpretation between the two systems is similar, where a higher score represents poorer mobility.

Mobility can be impacted by many factors. Age of the bird is important, and GS generally worsens as birds get older. Sørensen et al. (2000) found the percentage of birds in the upper categories of 4 and 5 were significantly higher at 7 wk of age as compared to 4 wk of age. Interestingly, when adjusted for body weight, no differences were noted.

Daylength and exposure to darkness may influence mobility. Brickett et al. (2007b) found only numerically better GS for birds with 12L exposure than with 20L, and this coincided with increased bone ash in the long scotoperiod birds. In both cases, GS averaged less than 1, indicating very good mobility within the entire flock, which may be why significance was difficult to demonstrate. Other research has not found a link. Sørensen et al. (1999), in a series of experiments using either 8, 16, 21 or 23 h of light per day, found no association between GS and lighting program unless an adjustment was made for body weight. However, the credibility of this research is limited by the fact that, in each of the four experiments that were completed, only two lighting programs were compared. Because of the high potential for bird source, bird

environment, disease status, and other factors to affect bird mobility, it is not accurate to compare and summarize across all trials.

The relationship of gait score to bird welfare is not linear with increasing gait score. If the gait of a bird is not perfect — in other words not a “0” on the GS scale — it does not mean that the welfare of the bird is reduced. However, there are benchmarks that help to understand the relationship of GS and bird well-being. Danbury et al. (2000) allowed lame birds to self-select an analgesic feed. Birds falling in the GS3 category chose to eat the analgesic feed, indicating that, at this point, they are masking pain by choice. McGeown et al. (1999) also found that walking of lame birds in the upper or >3 GS categories improved when an analgesic feed was fed, further supporting that pain is associated with moderate to severe lameness in broilers.

Changes in behaviour can also provide insight into bird welfare. Weeks et al. (2000) observed the behaviour of broilers with various gait scores and found that birds rested more and stood less as gait score increased. Higher gait scores also led to less time spent in comfort or maintenance behaviours, and birds fed less frequently but for longer periods of time. Birds in the GS categories of 0 to 3 spent the same total amount of time feeding. The combination of analgesic feeds and behavioural observations in these works show that birds in GS categories above 3 are in pain as a result of leg weakness issues, leading to reduced well-being.

1.3.3.2. Metabolic health

Another of the primary purposes for including darkness in a lighting program has been the reduction in metabolic disease (Classen et al., 1991; Lewis, 2006). Metabolic disease generally comprises a large portion of broiler flock mortality and has recently been reviewed (Julian, 2005). These diseases represent a financial cost to the industry because of the mortality levels, poorer growth rates, and higher incidence of condemnations at the processing plant (Julian, 1993). Once again, growth rate has been linked to higher levels of metabolic

disease and the reduction in growth rate seen when darkness is given to birds early in life has been suggested to improve bird health.

For example, Suh et al. (1998) provided broilers with near-constant, increasing or intermittent lighting programs, and found a reduction in Sudden Death Syndrome (SDS) in both flocks exposed to darkness. Final body weights did not correspond to the incidence of SDS, once again suggesting that it is the time of growth reduction that impacts the incidence of metabolic disease. The importance of timing of growth rate reduction, rather than final weight, on metabolic disease has also been shown using feed restriction early in life (Robinson et al., 1992).

Other research has also found a reduction in metabolic disease when darkness is provided to broiler chickens. In broilers exposed to 12, 16 or 20 h of light per day, a linear relationship was found between daylength and the levels of metabolic disease and, in particular SDS and ascites (Classen, 2004). In turkeys, levels of spontaneous cardiomyopathy declined when an increasing or decreasing program was used compared to a constant lighting program (Classen et al., 1994).

There are also other theories as to why darkness reduces metabolic disease. Exercise might be important, with more exercise improving cardiac function. Providing more darkness to broilers results in more activity and, as a consequence, more exercise (Bayram and Özkan, 2010). Longer periods of darkness reduce metabolic rate during the dark period, which in turn reduces pressure on bird metabolism (Classen et al., 2004).

1.3.3.3. Ocular health

Circadian rhythms have been suggested to be important in the growth of the eye. Eyes grow during the light period and stop growing during the dark. This is related to the production of melatonin and the expression of the melatonin receptors related to eye growth (Rada and Wiechmann, 2006). Therefore, the exposure to constant light disrupts the rhythm of eye growth, resulting in eyes that are abnormally large with an abnormal shape (Li et al., 1995).

Problems with eyes appear to be more severe in turkey flocks, which have a longer life span than broilers. Turkeys exposed to constant light have shown a higher incidence of abnormally large eyes (buphthalmos) as well as increased ocular pressure, cataracts, flattening of the cornea, retinal detachment and blindness (Barnett et al., 1971; Davis et al., 1986). The increased size is attributed to an accumulation of fluid, which results in increases in intraocular pressure (Smith et al., 1969). This can lead to corneal thickening, retina damage and cataract development (Li et al., 1995). If the pressure is increased, there is a potential that pressure is placed on the optic nerve, which would be uncomfortable or painful for the birds. All of these factors can play a part in the reduction of welfare for the bird.

Because of the relatively short life span of the broiler, it is more difficult to determine the welfare implications of constant light on eye health. However, relatively rapid changes in eye morphology as a result of constant light suggest that it is a welfare concern. In turkeys, even the exposure to one week of constant light resulted in flattening of the cornea, which can disturb the bird's ability to visually focus (Stone et al., 1995). Li et al. (1995) found damage to broiler eyes in as little as 10 d of constant light. These birds had higher levels of hyperopia, which after 11 wk was even more advanced. In other work, Li et al. (2000) found that exposure to less than 4 h of darkness resulted in significantly larger eyes. Changes such as these have been attributed

to the cessation of the melatonin rhythm, which impacts the growth of the eye (Li and Howland, 2003).

1.3.3.4. Foot pad lesions

Foot pad dermatitis is a skin lesion that occurs on the bottom of the foot of a bird. The lesions result from a breakdown of fecal matter in the litter, releasing ammonia and causing a lesion or a burn. In severe cases, there is no doubt that the lesion becomes uncomfortable or even painful (Bradshaw et al., 2002), leading to a reduction in welfare. Little work has pointed to lighting programs as a management factor that can be used to control foot pad lesions, but they may be part of a package to help reduce the severity. Kristensen et al. (2004) reported that any management practice that can increase bird activity can help improve the litter quality, which, in turn, results in less damage to the foot pad. Because behaviour varies with darkness exposure, then lighting programs may have an impact.

1.3.3.5. Immune function and stress

Exposure to darkness can impact immune function and stress (Abbas et al., 2008), and evidence for this exists with regards to a number of measurements. Zulkifli et al. (1998) found a higher heterophil-lymphocyte ratio in blood from birds raised on constant light as compared to those on 12 h of darkness exposure, suggesting higher stress levels in the constant lit birds. Møller et al. (1999) used fluctuating asymmetry (lack of bilateral symmetry within an organism) to judge immune function and stress, and found that constant lit birds showed higher fluctuating asymmetry compared to those with darkness exposure. Interestingly, they also found a positive association between asymmetry and tonic immobility, a technique used for fear assessment. In other research, both fluctuating asymmetry and higher heterophil-lymphocyte ratios were found in birds with constant light exposure as compared to those on 14L per day (Campo et al., 2007). Abbas et al. (2008) compared birds on intermittent programs to those on long daylength

programs and found an increased activation of T and B cell proliferation, suggesting better immunocompetence with darkness exposure. Interestingly, this research also found higher heterophil-lymphocyte ratios in the blood from the birds on the intermittent program. It is possible that the small group numbers and the use of an intermittent rather than constant darkness exposure used in this work contributed to this contrary finding.

Using quail as a subject, Moore and Siopes (2000) found a reduced cellular and humoral immune response when birds were exposed to constant light as opposed to those given 8 h light per day. In a second part of this study, exogenous melatonin was given to the birds, which resulted in an improvement in the immune response of the birds exposed to constant light. Similarly, constant light reduced T and B cell activity in broilers in comparison to birds raised on an intermittent program, with a greater response for T cells (Kliger et al., 2000).

1.4. Impact of darkness on behaviour

Welfare is often difficult to measure, but behaviour can be one of the strongest indicators of animal welfare available to scientists (Duncan, 1998, 2005). Behaviour can aid in the interpretation of an animal's feelings, both positive (such as playful behaviours, comfort behaviours and exploratory behaviours) and negative (including frustration, fear or pain). In addition, some behaviours are driven by strong motivations, and the reduction or the elimination of the performance of those behaviours is indicative of reduced welfare (Duncan, 1998). Dawkins (2004) reports that there are two primary questions that can define welfare: are animals healthy, and do they have what they want? Again, behaviour can help answer these questions.

Commercial poultry barns do not resemble the environment that the birds' wild ancestors lived in but, interestingly, the behaviours expressed by commercial broilers remain generally the same (Jurkevish and Grossmann, 2002). Because the environments are so different, it could be

expected that the time spent performing various behaviours would differ but not necessarily mean a reduction in welfare. However, if the incidence of a strongly motivated behaviour is reduced, it signals a welfare reduction (Dawkins, 1990; Duncan, 1998; Prescott et al., 2003). This is also true of behaviours that are necessary for functional living or are health dependant.

Observing behaviour is simpler now than ever before because of new technology. The use of cameras, especially those with infrared capabilities, allows the observation of animals with no human influence (Dawkins, 2004). Behaviour can be complex, and determining what the action of a particular behaviour means must be done with care, as a particular behaviour may have more than one meaning. This suggests that behaviour should be used as part of a package in the determination of welfare. In order to use behavioural assessments, it is important to have an understanding of what behaviours are normally performed, what the motivational factors are for a particular behaviour, and what the function the behaviour serves.

The behaviours that can be studied as a package to aid in welfare determination are many. Abnormal behaviours include stereotypical behaviours, which are repetitively performed behaviours with no apparent function (Broom, 1988). Expression of these behaviours indicates an abnormality and a reduction in welfare. Lethargy or lack of responsiveness to particular stimuli can signal an animal's inability to interact or cope with its environment (Broom, 1988).

Nutritive behaviours, including feeding and drinking, are necessary behaviours (Duncan, 1998), and the appetitive phase of these behaviours likely includes walking, scratching or foraging (Duncan, 1998). Broilers have a large motivation to feed, and frustration may be demonstrated when feeding is restricted (Bokkers et al., 2004). Although a reduction in highly motivated behaviours often indicates reduced welfare (Dawkins, 1990; Duncan, 1998; Prescott et al., 2003), the reduction could be due to a factor that is independent of welfare. Dawkins (1990) has pointed out that a reduction in nutritive behaviours could also be indicative of a

particular stage in an animal's life, so care must be taken with the interpretation of these behaviours. Drinking behaviour also changes as a bird ages, with bouts becoming fewer in number but longer in duration (Ross and Hurnik, 1983).

Locomotory or exercise behaviours can be indicative of skeletal health. Exercise is vital in bone health (Robbins et al., 1984), and both walking (Guadalupe-Grau et al., 2009) and standing (Chad et al., 1999) have been shown to provide enough load-bearing exercise in humans to be a contributing factor in bone health. This is likely true in birds as well, so the expression of these behaviours is important. A lack of movement can be indicative of negative welfare for other reasons as well, as it may indicate a lack of motivation to move or to reach other vital areas, such as feeders or waterers (Bokkers et al., 2004). Inactivity can also increase the incidence of hock and paw burns (Deaton et al., 1978; Gordon, 1994; SCAHAW, 2000). Running can be considered a play behaviour (Fraser and Broom, 1990), which will be discussed below. Fraser and Broom (1990) have termed extreme lethargy to be abnormal for broilers, and have suggested that neural malfunctioning might play a part in the development of this condition. This is important for a number of reasons, including the reduction or elimination of important behaviours and reduced bird reactivity. Reactivity or reflex action is generally the first response that animals have to stressful or dangerous situations (Fraser, 1988), so a reduction can again indicate reduced welfare. The issue of neural malfunctioning will be revisited in the section of sleep deprivation. It is of interest to note that inactivity is one of the reasons producers choose to use constant or near-constant light, as catching birds at the time of shipping is easier if birds are lethargic.

Comfort behaviours are typically performed when an animal is in a satisfied or comfortable state, so it is often thought that a reduction in these behaviours indicates an unsatisfied state (Delius, 1988). Many body maintenance behaviours fit in this category,

including preening (involved in feather maintenance (Hurnik et al., 1985; Duncan, 1998)), stretching (important in the maintenance of functional joints and muscles (Hurnik et al., 1985; Fraser and Broom, 1990)), and dustbathing (involved in feather maintenance (Hurnik et al., 1985)). Each of these behaviours is interesting in its own way. Preening, for example, is necessary for integument care and, therefore, the time between bouts cannot be too long (Delius, 1988). The timing of preening is usually when other needs have been met (Delius, 1988), but this behaviour can also be performed as a displacement behaviour (Delius, 1988). Hence, care must be taken with behaviour interpretation. Some comfort behaviours may be performed at inappropriate times, and can indicate stress or frustration (Duncan, 1998). Mills (2010) found increasing levels of scratching and preening in a flock of birds that was infested with parasites, which clearly is not a positive welfare situation. As mentioned above, it is also important to understand the motivation behind behavioural enactments in order to understand their importance. An example of this is dustbathing. Dustbathing is important in integument care, but it appears to be motivated by both external and internal factors (Duncan, 1998).

Play and exploration are indicative of positive emotion in birds (Duncan and Mench, 1993). Luxury behaviours can include running and foraging (Duncan and Mench, 1993; Duncan, 1998). It has been established that birds do have a motivation to explore and to forage (Fraser and Broom, 1990; Wood-Gush and Vestergaard, 1991; Newberry, 1999; Shields et al., 2004), so a reduction in these behaviours is likely indicative of poor welfare.

Welfare assessment can be aided by understanding positive behaviour but also by understanding negative behaviour. In birds, fear represents a state of emotional suffering (Jones, 1996). It can also lead to other states of welfare reduction, including tramping, clawing or smothering (Jones, 1996; Ghareeb and Böhm, 2008). Furthermore, it is thought that high fear levels result in the inhibition of many other motivational systems, with high fear levels

reducing the expression of important behavioural acts (Jones, 1996). For poultry, it appears that the highest fear levels result from either a sudden change in environmental cues or human contact (Jones, 1996). Fear is difficult to assess, but Jones and Faure (1981) have developed a test termed “tonic immobility” (TI) and “latency to rise”, which attempts to measure the flight and fight syndrome found in birds. Research such as that performed by Campo and Carnicer (1994) have strengthened the power of using TI by associating high levels of corticosteroid production with an increase in tonic immobility times. Although research into the behavioural patterns of broilers on different daylengths has been very limited, fear has been examined. Møller et al. (1999) and Campo et al. (2007) both found fear levels to be higher when birds were raised in constant light environments than when exposed to 14L:10D.

Analysis of vocalization has become a large tool in behavioural assessments (Manteuffel et al., 2004), and it can be helpful in understanding motivation and animal reactivity (Fraser, 1988). It is a communication tool and can be impacted by various parameters, including environment and animal awareness (Manteuffel et al., 2004).

Behaviour is generally not considered when producers choose a particular lighting program (Mauldin and Graves, 1984), but previous research has indicated that daylength can have an impact. Once again, contradictions are present particularly with reference to bird activity. Simons and Haye (1978) reported that activity levels were similar when darkness was included in a lighting program as compared to constant light programs. However, it is important to realize that this statement was made based on data collected only four times per day, from birds housed in battery cages rather than on floor pens similar to production facilities. Other research shows that darkness increases activity. Sanotra et al. (2002) compared birds exposed to 8 h of darkness per day to those given constant light, and found that activity was higher with darkness exposure. Again, these data were collected twice per day during the photophase only.

1.5. Circadian rhythms and sleep in poultry

1.5.1. Circadian rhythms

The study of chemical, biological or behavioural rhythms that occur in animals is termed chronobiology. Many of these rhythms occur with a frequency of approximately 24 h, although other time lengths exist as well. Many rhythms are centred on daily, tidal, lunar or even annual cycles (Menakar, 2002), and external environmental cues often trigger the cycles and maintain them within a specific time schedule. One of these primary triggers, called zeitgebers, is light, particularly dawn and dusk (Fleissner and Fleissner, 2002).

Circadian rhythms are important for the well-being of an animal, and the presence or lack of these rhythms can be used as an indicator of welfare (Bessei, 2006). It is important that individual animals are able to synchronize external and internal cues, including behavioural, physiological or metabolic functions of the body (Ohta et al., 2005; Kohsaka and Bass, 2006). For example, we know that feeding occurs in a rhythmic fashion primarily during the light period (May and Lott, 1994), and that rest occurs primarily during the dark period (Appleby et al., 2004). These are obviously different rhythms. If these rhythms were not present, then feeding might occur during the typical rest period, resulting in an interruption of sleep. When synchronized, this generally does not occur. When an organism does not follow a 24 h pattern, they are said to be “free-running” or an animal that has a daily rhythm which no longer follows a specific time clock. It is also important that a group of animals be synchronized with each other with regards to circadian rhythms, and this might involve their own species or a combination of species. For example, a very interesting and recent paper published in *Nature* (O’Donnell et al., 2010) noted synchrony must exist even between malaria parasites and their host organism. If this synchrony does not exist, then the viability of the parasite is reduced.

Melatonin is an important neurotransmitter in the body that functions to maintain and regulate many circadian rhythms (Brandstätter, 2002). It is vital in many physiological functions and also has an impact by modulating sleep, particularly via its effect to induce slow wave sleep (SWS) as has been shown in pigeons (Phillips and Berger, 1992). Melatonin is produced in a circadian rhythm fashion in either the pineal gland or retina of birds, with the peak in production occurring during the dark period and the valley in the day period (Pang et al., 1996). It plays a major part in the development of the bird circadian clock, and elimination of the melatonin production systems (primarily the pineal gland) can result in desynchronization of circadian rhythms (Gwinner et al., 1997). The effect of light on melatonin production is significant, as light is able to repress the production of melatonin (Appleby et al., 2004); this explains why melatonin production peaks during the night (Pang et al., 1996).

Continuous light has been shown to disrupt circadian rhythms. The elimination of darkness can be disruptive to the behavioural and physiological functioning of an animal. For example, young mice exposed to continuous light early in their lives have disrupted physiological rhythms that never recover even if given darkness later in life (Ohta et al., 2006). This has also been demonstrated in hospitals, where infants kept in isolators or rooms with constant light develop disrupted sleep rhythms (Mirmiran and Ariagno, 2000). Both of these research reports demonstrate the negative impact of constant light on the young. In the poultry industry, constant or near-constant light is commonly provided for young chicks up to the first week of life, at which time lighting programs, if used, are put in place. If disruption of these rhythms occurs with long photoperiods at this age, then perhaps maintaining young chicks under this type of lighting program is a mistake.

Behavioural research has indicated that daylength affects circadian rhythms in broiler chickens. Locomotory activity, and activity in general, ceases to follow a rhythm under constant light (Reiter and Bessei, 2002). May and Lott (1992) found that broilers raised on constant light lack the feeding rhythm found in birds given a night period. Disruption to behavioural rhythms with the use of constant light as compared to a photoperiod program with darkness inclusion (16L:8D) was also shown by Ferrante et al. (2006). However, bird densities differed between the two groups, with birds under constant light housed at a heavier density than those with darkness, which could impact behaviour. These data help strengthen the argument that light is the primary zeitgeber in establishing biological rhythms.

When examining feeding behaviour in particular, a strong rhythm is generally present under a day-night photoperiod program, and light again appears to be the primary zeitgeber. Generally, birds prefer to feed during the daytime (Savory, 1980), and the largest percentage of feeding usually takes place immediately after lights come on, and again in anticipation of lights going off at the end of the day (Savory, 1980). This final feeding period is important, as this fills the crop prior to the scotophase to provide nutrients for digestion during that time (Savory, 1980). It is important to realize that time spent feeding during the day is partially dependant on environmental characteristics. For example, eating a mash diet requires more time than eating a concentrated pelleted ration; thus, birds eating a mash feed will have a different rhythm than those eating pellets. Limited feeder space in a broiler flock will also alter feeding patterns, as all birds likely cannot feed when they would like to. These factors should be recognized when behavioural observations are performed, and might be a factor when deciding on a lighting program.

1.5.2. Sleep

Sleep and its functions are not well understood in birds, partially because of the difficulty and expense in conducting the experiments, and partially because data interpretation is difficult (Blokhus, 1983, 1984). Sleep occurs as a circadian pattern (Jones et al., 2008) and the presence of light and dark within a day is one of the main regulators in establishing this pattern (Fraser and Broom, 1990). The science of sleep has been anything but conclusive with disagreements over its function in the literature. It is generally agreed that sleep in a variety of species alters metabolism, which allows tissue regeneration to take place (Everson, 2005). It also functions in energy conservation, recovery of memory or brain function, ability to handle stress, and health (Blokhus, 1983; Everson et al., 1994; Sejnowski and Destexhe, 2000; Boerema et al., 2003; Everson and Crowley, 2004; Everson, 2005; Malleau et al., 2007).

Lack of sleep, or sleep deprivation, can be a negative influence on the welfare of an animal (Blokhus, 1983). It can cause a wide range of negative actions within an organism, and much centres around brain function. In general, lack of sleep results in a reduction in alertness and productivity of an animal (Boerema et al., 2003). Rats exposed to repeated awakenings, which leads to severe sleep deprivation, showed physical and mental symptoms including muscular weakness, footpad lesions, reduced brain functioning, pathological disease, and death (Rechtschaffen et al., 1983). Everson et al. (2008) found sleep-deprived rats to be stressed, as indicated by high transfer levels of neutrophils into liver tissue and lung tissue. Seemingly in contrast, there was no corresponding increase in corticosterone levels, which are often used as a measurement of stress. The authors believed that their findings suggest an early stage of an inflammatory response in these animals, which is likely the start of the disease process and may explain why sleep deprivation can lead to a state of unhealthiness in animals. In humans, regardless of how sleep deprivation occurs, reactivity suffers to such a degree that those with severe sleep deprivation are actually less reactive than when blood alcohol levels are

approximately 0.05 (Powell et al., 1999, 2001). Sleep deprivation causes neural activity to suffer or be reduced (Fraser and Broom, 1990), which is the result of reduced synaptic activity in the brain (Chen and Kushida, 2005). Poor reactivity and reduced brain function could potentially manifest itself as individuals who appear lethargic and do not express behaviours to the same degree as those with normal sleep patterns. Because the symptoms of sleep deprivation can be so substantial, it should be one of the deciding factors when choosing a lighting program for broilers (Blokhuis, 1983).

Sleep deprivation also appears to have a strong negative impact on weight control and digestive efficiency in humans. Sleep deprivation increases levels of hunger through a reduction of leptin and increase in ghrelin levels, and obesity and diabetes can be a result (Copinschi, 2005). It also impacts how nutrients are used in the body. Sleep deprivation reduces the ability to clear glucose or carbohydrates from the blood (indicative of pre-diabetic state and can lead to higher levels of cardiac disease), reduces thyroid functioning due to lower levels of thyrotropin production, and results in higher activity levels of the sympathetic nervous system (Speigel et al., 1999; Copinschi, 2005). In rats, food intake also increases with small amounts of sleep deprivation but no body weight gains are noted, suggesting that nutrient digestibility is reduced (Everson et al., 1994).

Why does this happen? Sleep debt causes a disruption of the functioning of the hypothalamus, and growth hormone production no longer responds in its typical pulsatile fashion. The result is a depressed level of growth hormone (Krieger, 1980), IGF1, prolactin and leptin (Everson and Crowley, 2004). Interestingly, a reduction in melatonin production has a similar impact on these hormones (Pang et al., 1996; Zeman et al., 1999; Zawilska et al., 2007). When looking at the materials and methods of many sleep studies, subjects (rats) are kept awake by placing them on a disk above water, or on a treadmill; generally, it is not stated that

darkness is provided, so continuous light is likely used. Most human work is done with visual stimulation to maintain wakefulness so again light is provided. It is possible that the lack of melatonin production because of constant light confounds the interpretation of sleep deprivation in these studies.

Sleep deprivation also appears to lessen an animal's ability to react to stress, which in turn negatively affects health (Blokhuys, 1983; Mueller et al., 2008). An example can be found in rats. One of the typical reactions to a physical stressor is an increase in the production of adrenocorticotrophic hormone (ACTH), which leads to the production of glucocorticoids. A period of 48 h sleep deprivation resulted in increased levels of both ACTH and corticosterone in rats, but these levels returned to normal after a recovery rest period (Meerlo et al., 2002). In a subsequent stress handling test to determine the rats ability to handle additional stresses, it was noted that the animals subjected to sleep deprivation had a lower ACTH production than the control rats, suggesting a reduced ability to respond to stress (Meerlo et al., 2002). Rechtschaffen et al. (1983) found that rat adrenal gland size increased in chronically sleep deprived rats, which is an indication of stress. These same rats had higher incidences of stomach ulcers and higher mortality levels. The latter group of animals were tested under constant light, and it is possible that these symptoms might be a result of a loss of melatonin circadian rhythms (Moore and Siopes, 2000; Kliger et al., 2000).

Sleep in birds may be different than in other animals, as birds have the ability to sleep unihemispherically while many other animals sleep with both cerebral hemispheres at the same time (Ayala-Guerrero et al., 2003). However, after a period of sleep deprivation birds migrate towards binocular sleep, which likely reduces alertness and predator awareness. Sleep in birds is suggested to have two main components, with each important for different reasons.

Quality sleep in birds is composed of two primary stages. Blokhuis (1983) has identified these as slow-wave sleep (SWS) and fast-wave sleep (FWS). One of the confusing sectors of the current literature appears to be differing terminology for similar states. For example, Rattenborg et al. (2009) also refers to the two primary stages of sleep but, in their case, these are termed SWS and rapid eye movement (REM) sleep. Hence, FWS and REM sleep are the same stage. Similarly, Chen and Kushida (2005) discuss the presence of non-rapid eye sleep (nREM), which is the same as SWS.

Confusing the literature even more are descriptions of the sub-stages that exist within these two primary stages. Once again terminology differs but, in general, appears to deal with the degree of sleep that occurs, particularly in the nREM phase of sleep (Chen and Kushida, 2005). Attempting to separate these phases of sleep with the use of an electroencephalogram (EEG) reading is very difficult, and is one of the reasons that sleep research is difficult to perform (van Luijtelaar et al., 1987; Coenen et al., 1988).

Stages of sleep are important, as they appear to have different restorative functions. Hence, all must be present for sleep to have its maximum efficiency. The phase of sleep changes as sleep progresses and, in laying hens, the very early stages of sleep are SWS. This stage is deeper sleep, with more large waves on the EEG pattern (van Luijtelaar et al., 1987). This lessens as the night progresses, and sleep becomes lighter (van Luijtelaar et al., 1987). FWS (or REM sleep) never occurs without SWS as a precursor (van Luijtelaar et al., 1987; Ayala-Guerrero et al., 2003). The functions of each vary as well. Serotonin production appears to be associated with SWS (Sabelli and Giardina, 1970), but FWS appears to be more important in stress adaptability (Blokhuis, 1983). In terms of memory restoration, both SWS and FWS sleep are important but for different memory types, likely through the production of different

memory neurotransmitters and neurohormones produced during each of the sleep phases (Born et al., 2006).

Sleep deprivation can result from a reduction in either the quality or the quantity of sleep (Chen and Kushida, 2005), which could both be affected by a lack of darkness and consequential disruption of circadian patterns (Manser, 1996). Although birds can sleep during the light phase of the day (Savory and Duncan, 1982; Ayala-Guerrero et al., 2003), Rattenborg et al. (2005) found that sleep was not as complete and suggested that this was attributed to the suppression of melatonin synthesis. Therefore, the quality of sleep is affected because all of the sleep stages are not present. Ookawa and Gotoh (1964) found differences in the EEG recordings of chickens sleeping in the dark or the light. They found that the birds sleeping during the dark showed waves consistent with both active and quiet sleep, while those sleeping in the light only showed waves consistent with quiet sleep. In contrast, Ayala-Guerrero et al. (2003) found that turkeys could reach REM sleep under constant light. Even if broilers can achieve all stages of sleep during the day, a lack of behavioural synchronization in a large flock exposed to constant light would likely result in sleep disruption of pen mates. The turkeys used by Ayala-Guerrero et al. (2003) were tested in a single bird chamber and so may have been able reach the latter stage of sleep.

Sleep fragmentation represents another type of sleep deprivation, and occurs with repeated awakenings (Bonnet, 2005). It can result in symptoms similar to total sleep deprivation (Bonnet, 1986; Chen and Kushida, 2005). In humans, even one night of sleep fragmentation can result in poor psychomotor performance, poor reactivity, and increased sleepiness. After 64 h of sleep fragmentation, symptoms are identical to total sleep deprivation. This likely occurs because, with repeated awakenings, a shift in brain waves occurs and appropriate time is not spent in SWS or REM sleep to satisfy sleep requirements (Jones et al.,

2008; Martinez-Gonzalez et al., 2008). Sleep fragmentation can also occur in a flock situation. If behavioural circadian rhythms are not synchronized under constant light, then there is no denoted rest period and birds that are attempting to rest will likely be continually interrupted. Hence, both quality and quantity of sleep could be reduced via sleep fragmentation.

It is also interesting that the nature of darkness provision can impact sleep. Coenen et al. (1988) compared birds on an intermittent program (15 min L:45 min D) to those raised on 14L:10D and found different EEG patterns during the dark period. Birds on the long scotoperiod reached the primary stages of sleep, noted on the EEG pattern by moderate to large slow waves. Those on the intermittent program were more restless and tended to be drowsy during the dark rather than asleep, as demonstrated by only moderate waves on the EEG pattern.

Often, preference tests are used to help understand what animals want in their environment. This research has been limited in poultry, but an interesting study was published by Malleau et al. (2007). In this work, the authors studied the importance of rest in young chicks, comparing those on a long day of 19.33 h to those on a simulated brooding cycle of 40 min light on followed by 40 min off. They found that chicks given the brooding light cycle took advantage of the dark cycles, and rested approximately 9 h more per day than those under the long photophase program. This indicates that rest is important for bird welfare, not only from a physiological and behavioural perspective as explained above, but also to facilitate the question of “what do they want?” (Dawkins, 2004).

1.6. Conclusions

The information presently available in the literature suggests that the use of constant or near-constant light results in poorer bird welfare although, again, there is disagreement in some respects. If we revisit the 5 Freedoms included in the introduction of this paper, we can see the areas that are an issue.

1. Freedom from thirst, hunger and malnutrition – by ready access to fresh water and a diet to maintain full health and vigor. Regardless of the photoperiod, broilers are generally given access to fresh water and food so this freedom is not denied as it is written. It could, however, if the Freedom was extrapolated. Some research programs have found an increase in leg weakness occurring under long photoperiods, and that these birds have difficulty reaching the feeders and waterers. If not culled, these birds could potentially starve or dehydrate.

2. Freedom from discomfort – by providing a suitable environment including shelter and a comfortable resting area. Regardless of the photoperiod, a suitable environment can be provided and therefore this Freedom will be met under any lighting program.

3. Freedom from pain, injury and disease – by prevention or rapid diagnosis and treatment. Long daylength increases mortality, and this has been one of the primary reasons for darkness to be used in a photoperiod program. It also has been shown to negatively impact leg weakness, which can be painful, and increase levels of metabolic disease. Hence, constant or near-constant light denies the birds this requirement.

4. Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of an animal's own kind. While space, facilities and company are not specifically related to photoperiod program used, this freedom is denied for other reasons. Sleep deprivation may result from long daylengths. Circadian rhythms may be disrupted, and some behavioural outputs vary. This area has not been well-documented with regards to daylength in broilers.

5. Freedom from fear and distress – by ensuring conditions which avoid mental suffering. There is evidence in the literature that this Freedom is also denied under constant or

near-constant light. Fear as measured by tonic immobility testing may be higher, and neural functioning may be poor due to the impact of sleep deprivation on the birds.

To conclude, there is evidence that the use of continuous light negatively impacts the welfare of broilers. The majority of research done in this area looks at small components of each of these factors, and has often involved the use of one lighting program as compared to another lighting program.

1.7. Objectives

The primary objective of this study is to establish the effect of daylength on the welfare of broiler chickens. Key to this investigation is the use of graded levels of daylength that permit the establishment of relationships between daylength and welfare criteria. Because welfare assessment is complex and multifaceted, a wide range of parameters will be assessed including growth rate, incidence of mortality, bird mobility as measured by gait score, incidence of foot pad lesions, behaviour, ocular health, and the presence of circadian rhythms of physiological importance. A second objective is to investigate the impact of daylength on broiler productivity and, for this, measurements include growth rate, feed intake levels, efficiency of feed conversion, and mortality and morbidity. Parameters for assessment of welfare and productivity are not mutually exclusive. A third objective is to provide data that can be used to model the impact of daylength on broiler welfare, flock productivity and the economics of broiler production.

1.8. Hypothesis

Daylength in broiler lighting programs affects bird welfare.

1.9 Publication note

A version of each of the four research papers in this thesis have been submitted for publication in peer-reviewed journals. In all cases, the journals have also been made aware that the paper has been included in a thesis.

The scope of this project was very large. It initially involved 3 graduate students – K. Schwean-Lardner, a second student working on the immunological aspect of daylength control, and a third student working on the muscle development aspect of use of daylength.

Although each paper is the work of K. Schwean-Lardner, each also has co-authors. Outlined below are the contribution areas of each co-author towards these papers:

H.L. Classen – Ph.D. Supervisor, expertise in poultry management and welfare,

B.I. Fancher – involved in the initial determination of the direction of research, also responsible for providing funding for the project,

S. Gomis – Veterinary Pathologist providing expertise in the etiology of disease,

A. Van Kessel – scientist involved in the initial discussions of the direction of the research – supervisor of the second graduate student working on the project,

S. Dalal – co-Ph.D student working on the immunological aspect of daylength control, and

B. Laarveld – scientist involved in the initial discussions of the direction of the research – supervisor of the third graduate student working on the project.

Preface to Chapter 2: Effect of daylength on production parameters in commercial broilers

The primary objectives in this work were to examine the impact of graded levels of darkness on welfare and productivity of broilers. The data in Chapter 2 focused primarily on production data, and included growth, feed intake, feed efficiency, and overall mortality as impacted by daylength, bird gender and genotype. While considered to be primarily “production” data, the unexpected growth rates under long daylengths, as well as the increased levels of mortality, were the first signal of reduced welfare in broilers found in this research.

Chapter 2. Effect of daylength on production parameters in commercial broilers^{*}

^{*} A version of this chapter has been accepted for publication: Schwean-Lardner, K., B.I. Fancher, and H.L. Classen. 2011. Impact of daylength on the productivity of two commercial broiler strains. *Br. Poult. Sci.* Accepted April, 2011.

ABSTRACT The impact of daylength on modern commercial broilers was studied in two experiments. In each experiment, four lighting programs (14L:10D (14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L)) were chosen to study relationships between broiler production and daylength. The first experiment was repeated in a block formation four times, with each block containing two full-room replications and including a total of 16,128 broilers. In addition to daylength, other main effects studied in this experiment were genotype (Ross x Ross 308 and Ross x Ross 708) and gender, and these variables were randomly placed within each room. Birds were weighed at 0, 7, 31/32 (d32), 38/39 (d39) and/or 48/49 (d49) d of age. Statistics included SAS General Linear Model to test for differences in light, genotype and gender, and interactions between the three main effects. Regression analysis was used to define relationships between productivity variables and daylength. The second experiment included two replications of each of the four lighting programs described above in a format similar to a commercial situation. The total number of birds in this experiment was 7,552, equally distributed into one of eight rooms, with each room containing equal numbers of male and female Ross x Ross 308 broilers. Data were compared using General Linear Model of SAS and regression analysis of SAS. In experiment one, body weights at d32 and d39 were related to daylength in a quadratic fashion, with the heaviest birds raised under the 20L photoperiod. The growth curve at d49 was similar in shape, and body weights under both 17L and 20L were higher than birds raised under 14L and 23L. In experiment 2, data were similar, although the quadratic relationship approached significance at d31 ($P=0.09$), and was not significant at d38. Feed consumption was highest to d39 or d49 under 20L in both experiments, and birds under near-constant light ate as much (d39) or less feed (d49) feed than birds given 17L. Mortality corrected or not, gain to feed efficiency was also impacted by daylength in a quadratic fashion, with the best efficiency

observed for broilers given the shortest daylength. Mortality increased linearly with daylength for all time periods in both experiments. Differences between genders were noted, including males growing faster and to heavier weights, improved feed conversion ratio and higher mortality. Genotype differences indicate that the Ross x Ross 308 strain grow faster and to heavier weights, are less feed efficient and have a higher mortality rate than do the Ross x Ross 708. The research reported in this manuscript demonstrates that daylength affects broiler growth, feed intake, feed efficiency and mortality, and that the response of these criteria is either quadratic or linear in nature.

Key words: photoperiod, light, dark, growth, efficiency, mortality

INTRODUCTION

Birds rely on vision more than any other sense (Prescott et al., 2003). In addition, light is the primary tool used by the body to entrain circadian rhythms (Marrus et al., 1996; Middleton et al., 1996; Rattenborg et al., 2005). However, these factors have rarely been a consideration when a decision is made about the use of lighting programs in broiler flocks. Constant or near-constant programs have been a traditional choice of photoperiod exposure, primarily with the thought that full or nearly-full access to light could provide constant visual access to feeders and waterers, which in turn would maximize feed consumption and growth. Although various lighting programs involving more darkness exposure have been studied and recommended, nearly continuous daylength is still common in commercial broiler production. The 1 hour (h) of darkness in the 23 light (L):1 dark (D) schedule has not been provided as a period for rest or sleep, but rather to allow birds to become accustomed to darkness during a potential power failure (Savory and Duncan, 1982).

A lack of darkness in many species has negative implications based on biological or physiological reactions. For example, the exposure of young mice to continuous light alters body circadian rhythms, and has a lasting effect on these rhythms throughout their life (Ohta et al., 2006). In humans, the exposure to continuous light for preterm infants in hospitals can contribute to disruptions in sleep rhythm and results in non-synchronized body rhythms (Mirmiran and Ariagno, 2000). This research demonstrates that rhythm development, and therefore disruption of the rhythm, starts at a very early age. If also true in birds, this could have important implications in newly hatched chicks, which are generally given long daylength lighting programs after placement in the brooding facility.

Lighting programs involving darkness exposure have been used for many years as a simple management tool in broiler production, primarily for a reduction in leg disorders, metabolic diseases and overall mortality (Classen and Riddell, 1989; Lewis et al., 1996; Brickett et al., 2007b). While the implications of adding darkness to a photoperiod schedule have been fairly consistent in terms of mortality and morbidity, the effect of darkness on growth and feed efficiency has been less consistent. For example, Morris (1967) found that growth and feed efficiency was maximized under continuous or near-continuous photoperiods. However, Buyse et al. (1994) compared near-continuous programs to either step-up or step-down lighting programs, and found growth on the near-constant program to be equal or poorer than that achieved with the changing or intermittent programs. They emphasized that since changing the photoperiod at 7 days (d) of age initially reduced growth rate, compensatory gain had taken place later to allow body weight to equal or exceed the near-constant program. Similarly, Rozenboim et al. (1999) found that birds raised to 42 d under 23L:1D were heavier than those with 8 h of darkness, but that by 49 d these differences had disappeared. Using more current genetic lines, which may react differently than birds in the older research listed above, Brickett et al. (2007a) found that adding 12 h of darkness reduced market body weight at 35 d as compared to using a photoperiod of 20L.

Much of the lighting work already mentioned has focused on comparisons of particular lighting programs rather than taking an experimental approach that permits prediction of the production response to daylength. Therefore, the objective of this research was to examine the effects of graded levels of daylength on production traits in broilers, potentially allowing the development of response curves to daylength in rapidly growing birds.

MATERIALS AND METHODS

Approval of all experimental protocols used in this work was granted 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.

Experiments

Experiment one. Four trials were conducted to study the effect of daylength, gender and genotype, and their interactions on production parameters in broilers. Minor differences between trials were found in final estimated housing density and market ages (Table 1).

All birds were given 23L:1D until 7 d of age and then four daylengths were tested (14L:10D (14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L)) until market age to allow regression analyses to be completed. Light intensity was 20 lux until 7 d of age and 10 lux thereafter. Light intensity was measured at the centre of the middle pen in each room (bird height) prior to placing chicks and again at light program initiation. A total of eight rooms were randomly allocated to the lighting treatments in each trial, allowing two room replicates per lighting program per trial, and eight replications per lighting program in the experiment. Lighting was provided with incandescent bulbs.

Within each of the eight rooms were 12 pens (2.3 x 2.0 m). Two strains were tested (Ross x Ross 308 and Ross x Ross 708), and broilers were housed sex separate. Each strain by gender subclass was replicated two or three times in each room.

Experiment two. One trial was conducted in which only lighting programs were studied.

Two rooms, each housing 944 mixed-sex Ross x Ross 308 broilers (equal number of males and females) in one pen, were randomly allocated to one of the four lighting programs as described in experiment one.

Housing and management

Chicks were hatched at a commercial hatchery, transported to the research facility and then randomly placed into experimental rooms. Supplemental heat was provided by hot water pipes and temperature was controlled thermostatically and gradually reduced from 35°C at d 0 to 22°C at 35 d. Feed, based primarily on corn and soybean meal, was provided by a commercial feed company and fed ad libitum in tube feeders (one tube feeder per pen in Experiment 1 and 12 per pen in Experiment 2; 0 to 24 d – 110 cm circumference; 24 d to market – 137.5 cm circumference). Birds were allotted 0.5 kilograms (kg) crumbled starter and 2.0 kg of crumbled grower per bird placed, and a finisher pellet (finisher 1) for the remainder of the experiment (Tables 2 and 3). For birds kept until 48/49 d, 1.6 kg per bird finisher 1 was followed for the remainder of the trial by finisher 2. Water was provided ad libitum through Lubing 4087 nipple drinkers, with six nipples per pen in Experiment 1, and 12 lines of six nipples per pen in Experiment 2.

Pens were bedded with wheat straw to a depth of approximately 7.5 to 10 cm. Clean litter was used in Experiment 1 – block 1 and 2, with rooms being cleaned and disinfected between flocks, and reused litter used in blocks 3 (second flock on litter) and 4 (third flock on litter).

Data collection

Data were collected to assess the effect of daylength, sex and genotype on broiler performance. Chicks were weighed on d0, and birds and remaining feed were weighed on d7, d31/32 (indicated as 32 d), d38/39 (39 d) and, for trials lasting longer, on d48/49 (49 d). From these data, average body weight, average feed consumption, and feed efficiency (gain to feed ratio) with and without mortality correction, were calculated. Birds were monitored on a daily basis. Any bird showing physical (loss of body weight, stance, feather position, comb color, inability to move) or behavioural indications of illness were culled as per University of Saskatchewan Standard Operating Procedure Guide. All routine mortality and culls were collected twice per day and necropsied for cause of death or morbidity (Prairie Diagnostics Services, Western College of Veterinary Medicine, University of Saskatchewan).

Statistical analyses

In experiment one, the main effects of sex and genotype as well as interactions between sex, genotype and lighting were analyzed using PROC GLM (General Linear Model) of SAS (SAS[®] 9.1., Cary, NC) as a 2 x 2 factorial nested within four lighting programs, with trial number used as a random variable block. Because block was significant, it was not removed from the model. When significant differences existed, Duncan's Multiple Range Test was used to separate means, with a probability of difference level set at 5%. The experimental model statement for this analysis was

$Y = \mu + P + S + G + L(R) + S*G + S*L + G*L + S*G*L + e$, where

Y is the observation from the dependant variable,

μ is the population mean for the variable,

P is the trial effect (random),

S is the sex effect (fixed),

G is the genotype effect (fixed),
L is the light effect (fixed),
R is room,
S*G, S*L and G*L are the two way interactions between specified variables,
S*G*L is the three way interaction between sex, genotype and light,
and e is the random error associated with the observation.

Experiment two was analyzed similarly, but with no sex or genotype main effects (hence no interactions) and no trial block.

In both experiments, relationships between the dependant variables and daylength were studied using PROC REG (Regression) and PROC RSREG (Response Surface Regression) of SAS.

RESULTS

Lighting

Growth rate. Chick weights were consistent across lighting treatments (42 g), and within each trial. In experiment 1, body weight responded to daylength in a quadratic fashion at 32 d and 39 d, with the maximum body weight occurring under 20L, which was confirmed by the ANOVA (Table 4). Birds achieved similar weights under 17 and 23L, while those raised on 14L were the lightest. While the regression analysis was not significant at 49 d ($P=0.16$), the ANOVA demonstrated that the heaviest body weights were achieved under 17L and 20L, and birds given the shortest daylength of 14L weighed the same as those given 23L. A similar shaped curve was seen in the second experiment but with less replication; differences only approached significance as a quadratic relationship at 31 d ($P=0.0903$). Again, regardless of age, the lowest

numerical body weight was seen in birds raised under short days and long nights (14L) and the heaviest under 20L.

Feed consumption. The feed consumption response to daylength is shown in Table 5. Throughout most periods, the response was quadratic, with maximum feed intake for 20L broilers. The regression analysis for 7-38 d period in experiment 2 only approached significance ($P = 0.0676$) for a linear relationship despite the response curve appearing to have the same shape (quadratic P value=0.1102). Birds under 20L consumed more feed than any other birds. However, an age-dependent shift occurred with feed consumption under other lighting programs. In experiment 1, the feed consumption ranking for treatments was 23L>17L>14L for 7-31 d. From 7-39 d, consumption of birds given 23L was equal to those given 17L, and both consumed more than birds given 14L. Finally, from 7-49 d, birds raised under 17L consumed more feed than did those under 23L, and both of these treatments still consumed more than birds given 14L. The pattern of feed intake over the periods measured varied with lighting program (Table 6). Earlier in life (7-32 d), birds on long scotophase programs consumed less feed in relationship to birds on short scotophases, but similar amounts than during later growth periods.

Feed efficiency. Daylength impacted feed efficiency (with (Table 7) or without (Table 8) mortality correction) during the 7-32 d period and the 7-39 d period in a quadratic fashion. Daylength affected 7-49 d gain to feed ratio both with and without mortality correction in a linear fashion with values decreasing with longer daylength. Examination of gain to feed ratio from 7 to 39 d shows that birds given 17L and 23L weigh the same at market, but 17L birds consumed less feed, and therefore are more

feed efficient. This is again shown during the 7-49 d period, where birds raised under 14L are similar in body weight to those under 23L, but are more feed efficient.

Mortality. Mortality and morbidity increased linearly with increasing daylength for all periods except for the 7-31 d period of the second experiment (Table 9).

Sex and genotype

The effect of gender and genotype on productivity parameters is shown in Table 10. The differences shown are expected, and will only be briefly discussed in this paper. Males grew larger, consumed more feed and were more feed efficient than the females. Males also had higher mortality than did the females. Differences were noted in the genotype comparison as well, which may reflect the selection pressure applied by the primary breeding company and specific target markets. In this experiment, the Ross x Ross 308 broilers were heavier, consumed more feed, were less feed efficient and had higher mortality than the Ross x Ross 708 genotype.

Interactions

Significant interactions between lighting program and gender for body weight, feed consumption and gain to feed ratio are shown in Table 11. Daylength affected males and females in a similar fashion with minor differences in magnitude of reaction causing the significant interaction.

Only one interaction was noted between daylength and broiler genotype and that was for feed consumption from 7 to 32 d (Table 12). The interaction was due to a difference of magnitude only.

Table 13 demonstrates the significant interactions between gender and genotype that arose in body weight, feed consumption over the 7-32 d and 7-39 d periods, and

gain to feed ratios (with and without mortality correction) from 7-39 d. Once again, the significant statistical reaction is caused by a shift in the magnitude of changes across genders and genotypes.

DISCUSSION

Daylength is important in many aspects of poultry production, and in particular, has been well thought out with regards to breeding birds because of its requirement in driving reproductive fitness. However, daylength, and hence exposure to darkness, can have an important impact on growing birds as well.

Birds prefer to eat and drink during the photophase period and therefore increasing exposure to darkness generally reduces feed intake. However, birds will eat during the dark if daylength is short or other environmental factors cause a change in feeding behaviour (Cherry and Barwick, 1962). The 14L:10D lighting program used in this work caused only small numbers of birds to feed at night time as determined by behavioural observation (Schwean-Lardner et al., 2006b) and, as a consequence, the lower body weights noted for birds raised on 14L are likely due to a restriction in feed intake. It makes sense then that as the daylength is increased, so are body weights, at least until the daylength reaches between 17 and 20L, depending on market age. As birds get older, an adjustment occurs in feed intake, and birds on longer scotophase programs consume similar feed amounts to those on short scotophase programs. The reasons for this are not understood, but could be influenced by an adjustment in behaviour in learning to anticipate darkness. Bird body weights also change, and birds exposed to darkness, even the long dark period of 10 h, are the same size as birds given near unlimited visual exposure to feeders and waterers.

At the other end of the daylength spectrum, it is interesting to remember that one of the primary reasons for using constant or near-constant photoperiods in broiler production is to allow unlimited visual access to feeders, hence inducing maximum feed intake and market body weight (Lewis and Morris, 2006). This is not the case in the present research where, regardless of age, birds raised under near-constant light are not the heaviest. This is more difficult to understand than the reduction noted under 14L:10D, as birds under near-constant lighting programs do have the visual opportunity to feed for the greater part of the day.

Examination of behavioural activity within this work can help to explain this finding (Schwean-Lardner et al., 2006b). Birds raised on near-constant light are lethargic and, averaged over a full 24 hour period or during the photoperiod alone, spend less of their time at the feeder and performing mobility behaviours, exploratory behaviours and comfort behaviours than do birds on other lighting programs.

Data from other research are not always consistent with regards to body weight and photoperiod, and it appears that earlier work often indicated that constant or near-constant light maximized growth at market. Sørensen et al. (1999) exposed broilers to a wider range of daylengths, including 8, 16, 21 or 23 h light per day in four experiments, and concluded that longer photoperiods resulted in heavier birds at 35 d in three of those experiments, with no impact in the fourth. However, this work did not directly compare all of the photoperiods, for example 21 and 23 h of light, so it may have missed differences that occurred between these lighting programs. Similarly, Ingram et al. (2000) compared birds raised under 23L and 12L, and found that birds were heavier with the longer photophase when grown to 6 wk of age. Similar results were found by Savory (1976), who compared constant daylength to a lighting program with 12 h darkness at 10 wk of

age. An interesting article by Boon et al. (2000b) indicated that long dark periods reduced protein synthesis in quail, hence body weight was higher for short scotoperiod programs. However, this work did not provide feed during the scotoperiod, and although birds do not typically eat during the scotophase, it is possible that protein catabolism was more rapid than protein synthesis because of feed restriction, not daylength. In a comparison of 12L vs 20L, Brickett et al. (2007a) found that the longer daylength produced heavier birds at 35 d of age, but a constant or near-constant photoperiod was not included. The current work indicates through a number of experiments and a large number of birds that near-constant light no longer maximizes growth, and results were similar in each experiment. However, there are factors that could have had some impact. Data in this trial clearly indicate that as birds get older they learn to adapt to darkness very well. Previous research includes birds of varying ages. As well, research in this project have compared graded levels of darkness rather than a direct comparison of specific lighting programs, which may also help to understand the biological impact that darkness has on birds.

As with body weight, it is understandable that birds exposed to shorter days consume less feed than those with slightly longer days. Interestingly, based on the similarity of body weights at 49 d of age between birds raised on 14L and those on 23L, it appears that broilers are able to adjust feed intake to shorter days. A decrease in the daylength from a long day during the early brooding stage to a shorter day used throughout the grow-out phase likely causes an initial slowing of the growth rate (Kühn et al., 1996); however, the birds develop the ability to adjust to the change, and actually perform better the longer the birds are kept. There could be multiple reasons for this, including behavioural adjustment of birds on longer scotophases to anticipate darkness, improved health with exposure to dark periods, and/or an improvement in mobility of

these birds allowing easier access to feeders. It is still unclear why birds raised under 23L have a reduced feed intake despite the nearly unrestricted visual access to feeders and waterers.

In this work, shorter daylength, or alternately a longer dark period, improved feed efficiency. Because longer daylength had a negative effect on mortality levels, the feed efficiency values with mortality correction show a larger difference than those without correction. Metabolic rates have been shown to be reduced during the dark period (MacLeod et al., 1980), and this reduction would result in improved feed efficiency during that period. Other physiological changes occur with darkness exposure as well that could also contribute. Melatonin is a hormone produced in both the chicken retina and pineal gland that is heavily involved in the development of circadian rhythms (Hau and Gwinner, 1994). It has also been shown that exogenous melatonin can rapidly result in sleep onset (Hishikawa et al., 1969; Bermudez et al., 1983). Endogenous melatonin peaks during the dark period and drops during the day, and the amplitude of the rhythms is more pronounced with longer night periods (Schwean-Lardner et al., 2010). There has been evidence that the addition of melatonin to the diet of broilers can result in a reduction in feed intake and an improvement in efficiency of feed conversion (Clark and Classen, 1995; Apeldoorn et al., 1999). The reason for the improvement in feed efficiency is unclear but could be related to a reduction in energy expenditure when exogenous melatonin is added to the diet (Apeldoorn et al., 1999). This may be associated with an altered endocrine status (e.g., T_3 and T_4) or, alternatively, melatonin may enhance glucose uptake in the jejunum (Osei et al., 1989). Melatonin production in the flocks used in this work was influenced by photoperiod (Schwean-Lardner et al., 2010), which has been seen in other research (Zawilska et al., 2007). This could explain

the relationships between daylength and feed intake as well as daylength and feed efficiency.

The data in this work confirm that exposure to darkness is still an important benefit with regards to mortality, which is one of the early reasons for using darkness exposure in broiler production (Classen and Riddell, 1989; Gordon, 1997; Suh et al., 1998). It is interesting to note that mortality level is often associated with rapid growth in broilers, but that did not occur here. More likely, it is the pattern of growth that is important, and early rapid growth as was seen in birds on near-constant light impacts mortality the most. As well, lack of dark period may result in the loss of restorative metabolism during the dark period, which could also be an important aspect. The highest mortality level was found in birds raised under 23L. These birds did not grow the fastest and, in particular during the 7-48/49 d period, were actually not bigger than birds raised under any of the other daylengths including the shortest, 14L. It has been shown that broilers given constant or near-constant light grow faster earlier in life and that exposure to darkness restricts growth at this early stage; however, compensatory gain occurs later in life for the latter birds (Classen and Riddell, 1989; Buyse et al., 1996b). This compensatory gain has been attributed to an increase in growth hormone secretion (Buyse et al., 1997), which in turn can be stimulated by melatonin (Zeman et al., 1999). It is likely, then, that it is more correct to specify that mortality is affected by the time of rapid growth in broilers, i.e., the shape of the growth curve, rather than stating that it is affected by rapid growth alone. This is substantiated by the work of Robinson et al. (1992), who found early feed restriction of broilers to slow initial growth rate reduced mortality due to metabolic and skeletal disorders. Darkness exposure can also reduce mortality in other ways. Immune function in chickens can be improved with exposure to darkness (Abbas et al., 2008) and, as mentioned earlier, darkness induces sleep

(Rattenborg et al., 2005), which can alter metabolism and allow tissue regeneration to take place (Everson, 2005).

It is interesting to speculate why using a long photoperiod would result in a reduced body weight, a reduced feed conversion rate, but increased levels of mortality. As mentioned earlier, disruption of melatonin circadian rhythms could play a part. Melatonin has many important functions, and one is its positive influence in sleep initiation. While little is known about sleep in chickens, sleep is thought to be important in many aspects of life, including restorative tissue regeneration, energy conservation, and memory function; lack of sleep can lead to poor health and even death in many mammal species (Everson et al., 1994; Everson and Crowley, 2004; Everson, 2005). It is possible that constant or near-constant light results in sleep deprivation in broilers.

Research on sleep in free moving birds is difficult due to the need to use electroencephalographic equipment, which until very recently has involved limiting birds to very small spaces in order to record data. It is known that pigeons sleep primarily during the dark period, and that dark itself is important in inducing sleep (Rattenborg et al., 2005), likely due to the increased circulating melatonin initiated after darkness onset. So, it may be implied that lack of darkness could result in sleep deprivation in other birds as well. Sleep deprivation is not limited to a reduction in quantity of sleep and, therefore, can occur under other circumstances. A particularly interesting form is termed “sleep fragmentation”. This occurs when waking is repeated throughout the night, and results in reduction in the quality of sleep (Chen and Kushida, 2005), possibly due to a shift in the proportions and intensities of slow wave sleep (SWS) and rapid eye movement sleep (REM) in comparison to a sleep satisfied state (Jones et al., 2008; Martinez-Gonzalez et al., 2008). Because light is the principle zeitgeber for circadian rhythms in birds, a lack

of a definite night may impact the nature of these rhythms. It has been shown in mice that constant light has the ability to desynchronize the clock neurons within the body (Ohta et al., 2005), and these are thought to be important in the development of the 24 h rhythm that can be found in behaviour and physiological parameters. Birds raised under near-continuous or continuous light may not have a circadian rhythm in their behavioural patterns, or individual birds could follow free-running rhythms. With free-running rhythms, eating, drinking, and other behaviours such as walking may occur any time in the 24-h period, which likely disrupts other birds that are attempting to sleep in the light period. Therefore, it could be hypothesized that such behaviour would disrupt the sleep patterns of those birds attempting to sleep in the light period and result in sleep deprivation.

Sleep is important for many reasons. It has been implicated in the recovery of brain function, and lack of sleep can lead to reduced alertness and productivity (Boerema et al., 2003). Sleep deprivation has been found to have negative effects on growth rate and digestive efficiency. For example, in humans, a reduction in sleep over time (termed sleep debt) has been shown to lead to a negative impact on carbohydrate metabolism; blood glucose levels take approximately 40% longer to clear the body than in individuals with no sleep debt load (Spiegel et al., 1999). This work also demonstrated an inability to regulate hormone secretion in the body. Rats that are sleep deprived have been shown to become inefficient converters of feedstuffs, with food intake increasing but body weight suffering (Everson and Crowley, 2004; Hipólido et al., 2006). The degree of sleep deprivation does not need to be severe, and even a small degree of deprivation can result in higher feed intake with no change in body weight (Everson et al., 1994). There are a number of theories about why this occurs. Sleep deprivation causes a disruption at the hypothalamus level, with growth hormone production no longer responding in a pulse pattern and depressed levels of growth

hormone, IGF1, prolactin and leptin (Everson and Crowley, 2004) being produced. Interestingly, melatonin levels impact many of these and other hormones (Pang et al., 1996; Zeman et al., 1999; Zawilska et al., 2007). Sleep deprivation can also change plasma ghrelin:leptin ratios through declining leptin and increasing ghrelin levels, thereby affecting appetitive control (Copinschi, 2005). In rats, it reduces insulin, ACTH and corticosterone levels (Hipólido et al., 2006) and has been linked to reduced efficiency of the immune system (Everson, 2005). Birds raised on 23 h light consumed as much feed as did birds with a more extended dark period, yet did not show the same growth rate (hence a reduced feed efficiency). They also showed the highest level of mortality, and these effects are in agreement with the data found in rats.

Productivity in itself should not be used as a sole measurement of welfare, but unexpected and unexplained declines in productivity might be a signal that welfare is compromised. The research presented in this paper supports the concept that near-continuous light negatively affects bird welfare as indicated by a reduction in growth rate. Further, increasing mortality positively associated with daylength is also a clear indicator of poorer welfare with long to near-continuous lighting.

In conclusion, daylength impacts many important broiler production parameters and, as a consequence, is an important management tool. In general, shorter daylength initially decreases growth rate but body weight can be equal or superior at later market ages. Shorter daylength also improves feed efficiency and reduces mortality. Differences between 14L and 17L are minor, suggesting that it is not necessary from a production or a welfare point of view to use scotoperiods longer than 7 h. Near-continuous light unexpectedly reduces market body weight, increasingly so with older birds, and is not recommended from either a production or welfare standpoint. In

conclusion, productivity is impacted by daylength, and near-continuous light, particularly at older market ages, results in poor productivity. Long scotophase programs help to reduce mortality and improve feed efficiency, but may impact body weight at young marketing ages. The choice of a program should take specific situations into account.

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TABLE 2.1. Experiment and block details

Experiment One				
Block number	Total N	Age at Weight Measurement (d)	Final Estimated Floor Space	Pens/Room
1	5040	31, 38	24 kg/m ²	12
2	4464	32, 39, 48	30 kg/m ²	12
3	3712	31, 38	30 kg/m ²	8
4	2912	31, 38, 48	30 kg/m ²	8
Experiment Two				
1	7552	31, 39	32 kg/m ²	1
Summary:	Sum=23,680	(31,32), (38,39) or (48,49)		

TABLE 2.2. Composition of diets used in Experiment 1 – blocks 1 and 2 and Experiment 2

Ingredients: (%)	Starter	Grower	Finisher 1	Finisher 2
Corn	54.16	58.77	64.17	67.83
Soybean meal	37.60	32.70	27.50	24.80
Canola oil	3.25	4.00	4.00	3.35
Di-calcium phosphate	1.92	1.72	1.62	1.40
Limestone	1.56	1.41	1.36	1.24
Salt	0.35	0.37	0.36	0.33
Vitamin/Mineral premix ¹	0.19	0.19	0.19	0.14
Choline chloride	0.07	0.09	0.11	0.12
DL-Methionine	0.28	0.23	0.17	0.20
L-Threonine	0.02	0.01	0.00	0.03
L-Lysine HCL	0.17	0.11	0.11	0.15
Pro-Bond (Pea starch)	0.26	0.15	0.15	0.15
Sodium bicarbonate	0.22	0.20	0.21	0.22
Bio-cox 120	0.06	0.06	0.06	0.06
Rovomix E50	0.0004	0.0004	0.0004	0
Nutrients: (%)	Starter	Grower	Finisher	Finisher 2
AME (kcal/kg) ²	3050	3149	3200	3200
Crude protein	22.0	20.0	18.0	17.1
Calcium	1.00	0.90	0.85	0.76
Non-phytate phosphorus	0.50	0.45	0.42	0.37
Sodium	0.21	0.21	0.21	0.20
Arginine	1.51	1.36	1.20	1.121
Lysine	1.38	1.20	1.06	1.021
Methionine	0.62	0.55	0.47	0.481
Total sulphur amino acids	1.030	0.920	0.840	0.760
Threonine	0.88	0.79	0.70	0.691

TABLE 2.2. Composition of diets used in Experiment 1 – blocks 1 and 2 and Experiment 2

Ingredients: (%)	Starter	Grower	Finisher 1	Finisher 2
Tryptophan	0.31	0.28	0.24	0.223

¹ Supplied per kilogram of diet: vitamin A, 9425 IU; vitamin D, 3055 IU; vitamin E, 50 IU; vitamin K, 1.43 mg; thiamine, 1.95 mg; riboflavin, 6.5 mg; niacin, 65 mg; pyridoxine, 3.25 mg; vitamin B₁₂, 0.013 mg; pantothenic acid, 13.0 mg; folic acid, 1.1 mg; biotin, 0.163 mg; antioxidant, 0.081 mg; iron, 55 mg; zinc, 60.5 mg; manganese, 74 mg; copper, 5.5 mg; iodine, 0.72 mg; and selenium, 0.3 mg.

² National Research Council 1994.

TABLE 2.3. Composition of diets used in Experiment 1 – blocks 3 and 4

Ingredients: (%)	Starter	Grower	Finisher 1	Finisher 2
Corn	54.3	58.7	64.3	67.29
Soybean meal	37.5	32.62	27.47	25.40
Canola oil	3.3	4.15	4.10	3.35
Di-calcium phosphate	1.92	1.72	1.57	1.39
Limestone	1.58	1.40	1.39	1.24
Salt	0.361	0.368	0.346	0.330
Vitamin/Mineral Premix ¹	0.126	0.127	0.127	0.127
Choline chloride	0.018	0.086	0.098	0.119
DL-Methionine	0.324	0.264	0.234	0.198
L-Threonine	0.083	0.051	0.041	0.031
L-Lysine HCL	0.173	0.112	0.007	0.146
Pro-Bond (Pea starch)	0.150	0.150	0.150	0.150
Sodium bicarbonate	0.210	0.200	0.200	0.220
Rovomix E50	0.004	0.004	0.004	0.004
Nutrients: (%)	Starter	Grower	Finisher	Finisher 2
AME (kcal/kg) ²	3060	3163	3212	3200
Crude protein	21.7	19.7	17.6	17.1
Calcium	1.00	0.89	0.85	0.76
Non-phytate phosphorus	0.50	0.45	0.41	0.37
Sodium	0.211	0.210	0.201	0.20
Arginine	1.511	1.358	1.200	1.121
Lysine	1.380	1.200	0.980	1.021
Methionine	0.665	0.582	0.528	0.481
Total sulphur amino acids	1.030	0.920	0.840	0.760
Threonine	0.940	0.830	0.740	0.691
Tryptophan	0.309	0.275	0.241	0.223

TABLE 2.3. Composition of diets used in Experiment 1 – blocks 3 and 4

Ingredients: (%)	Starter	Grower	Finisher 1	Finisher 2
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¹ Supplied per kilogram of diet: vitamin A, 9425 IU; vitamin D, 3055 IU; vitamin E, 50 IU; vitamin K, 1.43 mg; thiamine, 1.95 mg; riboflavin, 6.5 mg; niacin, 65 mg; pyridoxine, 3.25 mg; vitamin B₁₂, 0.013 mg; pantothenic acid, 13.0 mg; folic acid, 1.1 mg; biotin, 0.163 mg; antioxidant, 0.081 mg; iron, 55 mg; zinc, 60.5 mg; manganese, 74 mg; copper, 5.5 mg; iodine, 0.72 mg; and selenium, 0.3 mg.

² National Research Council 1994.

TABLE 2.4. Effect of daylength on body weight (kg)

	Daylength (h)				SEM	Equation
	14	17	20	23		
Experiment 1						
32 d	1.644 ^c	1.677 ^b	1.738 ^a	1.703 ^b	0.0074	$Y=0.7395+0.0993X-0.0025X^2$
39 d	2.243 ^c	2.309 ^b	2.337 ^a	2.291 ^b	0.0112	$Y=1.1580+0.1211X-0.0031X^2$
49 d	3.197 ^b	3.268 ^a	3.272 ^a	3.170 ^b	0.0306	-
Experiment 2						
31 d	1.751	1.842	1.859	1.846	0.0191	-
38 d	2.341	2.362	2.390	2.385	0.0152	-

SEM = Standard error of the mean.

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

Regression analysis considered significant if $P<0.05$.

TABLE 2.5. Effect of daylength on feed consumption (kg) from light program initiation to listed age

	Daylength (h)				SEM	Equation
	14	17	20	23		
Experiment 1						
7-32 d	2.276 ^d	2.411 ^c	2.521 ^a	2.460 ^b	0.0128	$Y=0.1952+0.2248X-0.0006X^2$
7-39 d	3.424 ^c	3.592 ^b	3.719 ^a	3.629 ^b	0.0195	$Y=0.7480+0.2915X-0.0072X^2$
7-49 d	5.490 ^d	5.705 ^b	5.845 ^a	5.622 ^c	0.0510	$Y=1.2971+0.4692X-0.0122X^2$
Experiment 2						
7-31 d	2.215 ^b	2.393 ^a	2.454 ^a	2.407 ^a	0.0365	$Y=2.4374+0.0952X-0.1264X^2$
7-38 d	3.445	3.625	3.669	3.643	0.0402	-

SEM = Standard error of the mean.

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

Regression analysis considered significant if $P<0.05$.

TABLE 2.6. Effect of daylength on feed consumption (kg) for specific periods

	Daylength (h)				SEM
	14	17	20	23	
Experiment 1					
7-32 d	2.276 ^d	2.411 ^c	2.521 ^a	2.460 ^b	0.0128
32-39 d	1.134 ^b	1.164 ^a	1.171 ^a	1.144 ^b	0.0195
38-49 d	1.936	1.970	1.958	1.895	0.0510
Experiment 2					
7-31 d	2.215 ^b	2.393 ^a	2.454 ^a	2.407 ^a	0.0365
31-38 d	1.221	1.215	1.197	1.210	0.0106

SEM = Standard error of the mean.

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

Regression analysis considered significant if $P < 0.05$.

TABLE 2.7. Effect of daylength on feed efficiency with mortality correction (G:F^m) from light program initiation to listed age

	Daylength (h)				SEM	Regression Equation
	14L	17L	20L	23L		
Experiment 1						
7-32 d	0.660 ^a	0.645 ^b	0.635 ^c	0.637 ^c	0.0014	Y=0.8113-0.0163X+0.0076X ²
7-39 d	0.611 ^a	0.603 ^b	0.592 ^c	0.594 ^c	0.0014	Y=0.7411+0.0136X+0.0003X ²
7-49 d	0.553 ^a	0.546 ^b	0.536 ^c	0.540 ^c	0.0020	Y=0.5754-0.0017X
Experiment 2						
7-31 d	0.719	0.702	0.696	0.705	0.0038	-
7-38 d	0.635 ^a	0.610 ^b	0.614 ^b	0.618 ^b	0.0038	Y=0.6104-0.0072X+0.0163X ²

¹ G:F^m=(final period weight + kg of mortality weight-initial period weight)/period feed consumption.

SEM = Standard error of the mean.

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

Regression analysis considered significant if $P<0.05$.

TABLE 2.8. Effect of daylength on feed efficiency without mortality correction (G:F¹) from light program initiation to listed age

	Daylength (h)				SEM	Equation
	14L	17L	20L	23L		
Experiment 1						
7-32 d	0.651 ^a	0.638 ^b	0.625 ^c	0.627 ^c	0.0015	Y=0.8216-0.0179X+0.0004X ²
7-39 d	0.604 ^a	0.595 ^b	0.579 ^c	0.582 ^c	0.0014	Y=0.7451-0.0144X+0.0003X ²
7-49 d	0.547 ^a	0.537 ^b	0.518 ^c	0.523 ^c	0.0022	Y=0.5860-0.0030X
Experiment 2						
7-31 d	0.712	0.694	0.689	0.696	0.0042	-
7-38 d	0.629 ^a	0.602 ^b	0.605 ^b	0.607 ^b	0.0043	Y=0.6015-0.0092X+0.0167X ²

¹ G:F=(final period weight -initial period weight)/period feed consumption.

SEM = Standard error of the mean.

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

Regressions considered significant if $P<0.05$.

TABLE 2.9. Effect of daylength on mortality (% of birds placed) from light program initiation to listed age

	Daylength (h)				SEM	Equation
	14L	17L	20L	23L		
Experiment 1						
7-32 d	2.33 ^b	2.36 ^b	3.10 ^{ab}	3.54 ^a	0.141	Y=0.1434+0.1453X
7-39 d	2.93 ^b	2.84 ^b	4.28 ^{ab}	4.81 ^a	0.184	Y=-0.6478+0.2358X
7-49 d	3.63 ^b	3.87 ^b	7.11 ^a	6.55 ^a	0.423	Y=-2.1112+0.4001X
Experiment 2						
7-31 d	2.28	3.07	2.33	2.86	0.244	-
7-38 d	2.65	3.76	3.07	3.87	0.266	Y=-0.5067+0.0653X

SEM = Standard error of the mean.

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

Regression analysis considered significant if $P < 0.05$.

Table 2.10. Effect of sex and genotype, and interactions between sex, genotype and light on growth parameters

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	SEM
	Male	Female	308	708				
Body weight, 32 d	1.785 ^A	1.607 ^B	1.740 ^a	1.652 ^b	0.0157	NS	0.0001	0.0074
Body weight, 39 d	2.451 ^A	2.140 ^B	2.350 ^a	2.241 ^b	0.0036	NS	0.0473	0.0112
Body weight, 49 d	3.501 ^A	2.953 ^B	3.287 ^a	3.166 ^b	0.0021	NS	0.0357	0.0306
Feed cons, 7-32 d	2.525 ^A	2.309 ^B	2.506 ^a	2.328 ^b	0.0100	0.0439	0.0073	0.0128
Feed cons, 7-39 d	3.792 ^A	3.390 ^B	3.716 ^a	3.465 ^b	0.0261	NS	0.0243	0.0195
Feed cons, 7-49 d	6.050 ^A	5.281 ^B	5.842 ^a	5.490 ^b	0.0484	NS	NS	0.0510
G:F ^m 7-32 d	0.657 ^A	0.630 ^B	0.636 ^b	0.651 ^a	NS	NS	NS	0.0014
G:F ^m 7-39 d	0.614 ^A	0.586 ^B	0.593 ^b	0.607 ^a	NS	NS	0.0144	0.0014
G:F ^m 7-49 d	0.558 ^A	0.529 ^B	0.538 ^b	0.549 ^a	NS	NS	NS	0.0020
G:F 7-32 d	0.645 ^A	0.625 ^B	0.626 ^b	0.644 ^a	NS	NS	NS	0.0015
G:F 7-39 d	0.599 ^A	0.581 ^B	0.582 ^b	0.598 ^a	NS	NS	0.0253	0.0014
G:F 7-49 d	0.541 ^A	0.522 ^B	0.523 ^b	0.540 ^a	0.0477	NS	NS	0.0022
Mortality %, 7-32 d	3.70 ^A	1.96 ^B	3.35 ^a	2.32 ^b	NS	NS	NS	0.141
Mortality %, 7-39 d	5.01 ^A	2.42 ^B	4.35 ^a	3.07 ^b	NS	NS	NS	0.184
Mortality %, 7-49 d	6.88 ^A	3.70 ^B	6.79 ^a	3.80 ^b	NS	NS	NS	0.423

SEM = Standard error of the mean.

^{A, B} Means with common letters do not differ significantly ($P < 0.05$).

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

G:F=(final period weight -initial period weight)/period feed consumption.

G:F^m=(final period weight +mortality weight-initial period weight)/period feed consumption.

TABLE 2.11. Effect of the significant interaction between daylength and gender

	Age or Period	Sex	Daylength (h)			
			14	17	20	23
Body weight, kg	32 d	M	1.732	1.797	1.831	1.779
	32 d	F	1.556	1.602	1.664	1.626
	39 d	M	2.403	2.474	2.502	2.425
	39 d	F	2.084	2.144	2.173	2.157
	49 d	M	3.489	3.571	3.542	3.400
	49 d	F	2.906	2.965	3.002	2.940
Feed intake, kg	7-32 d	M	2.370	2.531	2.647	2.552
	7-32 d	F	2.181	2.292	2.396	2.367
	7-39 d	M	3.620	3.870	3.940	3.800
	7-39 d	F	3.227	3.378	3.498	3.455
	7-49 d	M	5.897	6.116	6.260	5.927
	7-49 d	F	5.083	5.295	5.431	5.318
G:F Ratio	7-49 d	M	0.557	0.551	0.552	0.533
	7-49 d	F	0.536	0.523	0.514	0.514

TABLE 2.12. Effect of the significant interaction between daylength and genotype

	Age or Period	Genotype	Daylength (h)			
			14	17	20	23
Feed consumption, kg	7-32 d	308	2.344	2.510	2.621	2.549
	7-32 d	708	2.207	2.313	2.421	2.370

TABLE 2.13. Effect of the significant interaction between gender and genotype

	Age or Period	Gender	Genotype	
			308	708
Body weight, kg	32 d	M	1.838	1.732
	32 d	F	1.642	1.573
	39 d	M	2.512	2.390
	39 d	F	2.187	2.092
	49 d	M	3.564	3.437
	49 d	F	3.011	2.896
Feed consumption, kg	7-32 d	M	2.626	2.424
	7-32 d	F	2.386	2.231
	7-39 d	M	3.931	3.653
	7-39 d	F	3.501	3.278
G:F ^m	7-39 d	M	0.606	0.622
	7-39 d	F	0.580	0.592
G:F	7-39 d	M	0.589	0.609
	7-39 d	F	0.574	0.588

Preface to Chapter 3: The effect of daylength on cause of mortality, leg health and ocular weight in broilers

The health of animals is one of the primary indicators of welfare. Hence, this data would not be comprehensive without examining the impact of daylength on mortality levels, causes of death, mobility in live birds (leg weakness) and changes to eye size as they are impacted by daylength. Chapter 3 focused primarily on the health of broilers under various lighting programs, and in Experiment 1, gender and genotype. It included the specific causes of mortality (of interest from a productivity and welfare standpoint), mobility scoring (welfare and production oriented) and ocular weights (welfare). Similarly to Chapter 2, this information is important from both a welfare and productivity standpoint.

Chapter 3. The effect of daylength on cause of mortality, leg health and ocular weight in broilers^{*}

^{*} A version of this chapter has been submitted for publication to Poultry Science: Schwean-Lardner, K., B.I. Fancher, S. Gomis, S. Dalal, and H.L. Classen. Effect of daylength on mortality, leg health and ocular health in broilers. Submitted October, 2011.

ABSTRACT An experiment was conducted to study the impact of daylength, gender and genotype (Ross x Ross 308 and 708) on mortality causes, mobility, footpad health and ocular size, with four blocked trials within the experiment. A second experiment examined the impact of daylength alone, total and cause of mortality, and daylength and gender on gait score. Four graded daylengths were chosen to allow the relationship between daylength and health parameters to be studied, including 14L:10D, 17L:7D, 20L:4D and 23L:1D. The primary statistical tools used to assess the daylength relationships were regression analysis, using Proc Reg and RSReg of SAS. Data were also analyzed as a 4 (lighting program) x 2 (gender) x 2 (genotype) factorial arrangement in experiment 1 and as a two-way factorial when sex was known (gait score, ocular weight) in experiment 2. Total, metabolic and skeletal mortality decreased linearly with increasing inclusion of darkness over the 7-32, 7-38 and 7-48 d periods. Infectious disorders were quadratically related to daylength in the 7-48 d period only, with birds under 20L having the highest level. Daylength was either linearly or quadratically related to average gait score in a positive fashion, and the incidence of birds falling in painful gait score categories increased linearly with increasing daylength. Average footpad lesion scores increased with increasing daylength at 28 and 35 d. The 23L photoperiod resulted in heavier eye weights than other lighting programs. Males had a higher mortality and morbidity rate and a higher average gait score than females. Average footpad score was lower for males than females at 28 and 35 d of age. Overall mortality was higher for 308 than 708 broilers and, as a result, levels of specific mortality causes were higher. Average gait scores were lower for 308 than 708 birds in two of the five time periods measured and footpad lesions were higher for three of the four measurement periods. To conclude, many aspects of broiler health improve with decreasing daylength.

Key words: daylength, mortality, gait score, footpad health, welfare

INTRODUCTION

There are many indicators of welfare in agricultural animals, and one of the most obvious is health. In broiler chickens, management practices, such as lighting programs, can impact health. Traditionally, constant light was thought to allow maximum feed intake and weight gain (Lewis and Morris, 2006) but it was found to also increase the incidence of metabolic and skeletal disorders (Manser, 1996) and reduce walking ability in the flock (Sanotra et al., 2002). Adding darkness to a lighting program has been used to reduce the incidence of these disorders and is thought to do so by reducing growth rate, particularly early in the production cycle, and/or through beneficial metabolic changes in the bird during the dark period (Classen and Riddell, 1989, 1990; Lewis and Perry, 1986; Brickett et al., 2007b). In addition, increased exercise that is associated with darkness addition may also positively influence skeletal health (Reiter and Bessei, 2009).

Recent research has added to the knowledge of lighting program effects and has questioned some of the above principles. Schwean-Lardner et al. (2011) found that birds given near-constant light were lighter than birds exposed to 4 hours (h) of darkness at a young age (31 days (d)). As birds got older, adding more darkness to the photoperiod program resulted in those exposed to 7 h being as heavy as or heavier than those birds exposed to near-constant light. In even older birds, those exposed to 10 h of darkness were heavier than those exposed to near-constant light. This same work confirmed that overall mortality was reduced by darkness exposure and corresponding shorter days. Darkness exposure was also found to have an important impact on bird behaviour, with near-constant light resulting in bird lethargy (Schwean-Lardner et al., 2006b) and a desynchronized flock rhythm in behavioural activity and melatonin output (Schwean-Lardner et al., 2010). It was hypothesized that a reduction in the quality and quantity of

sleep in birds given near-constant light could be a contributing factor in these production and welfare responses.

Sleep is believed to be involved in a wide range of factors, including memory development (Sejnowski and Destexhe, 2000), learning (Su et al., 2004), and regeneration of tissues and conservation of energy (Blokhuys, 1983; Malleau et al., 2007). Sleep deprivation also appears to lessen an animal's ability to react to stress (Blokhuys, 1983; Mueller et al., 2008), and the result can be detrimental to its health. For example, restricting sleep in rats resulted in significantly reduced ACTH response to a physical stressor, thereby lessening the animal's ability to respond to stress (Meerlo et al., 2002). This change to physiological functioning in the rat was also confirmed by Rechtschaffen et al. (1983), who found that sleep deprivation increased the size of the adrenal gland, increased the number of stomach ulcers, and increased mortality. The hormone most closely linked to sleep is melatonin, and it is the rhythmic production of this hormone that drives circadian rhythms in the body. The use of constant light appears to disrupt this rhythm, and that can have a negative impact on the health of a bird, such as reducing the effectiveness of immune responses (Kliger et al., 2000; Moore and Siopes, 2000).

The objective of this project was to determine if graded levels of daylength impact various measures of broiler health and well-being. Response criteria included the cause of mortality and culls, gait score, foot pad health, and eye size.

MATERIALS AND METHODS

Permission was granted for all experimental work by the University of Saskatchewan Animal Care Committee, with all procedures following the recommendations of the Canadian Council of Animal Care (1993) as listed in the Guide to the Care and Use of Experimental Animals.

Experiments

One experiment, which included four trial repetitions in a small pen environment, was conducted to study the impact of daylength, bird gender and genotype on health parameters of broilers. A second experiment using a large pen size model was performed to determine the impact of daylength alone on these parameters using only one genotype. Specific details of these experiments, including housing and management of birds, are included in Schween-Lardner et al. (2011).

Lighting programs were initiated in all experiments at 7 d of age. Prior to this time, birds were housed under 23 h light and 1 h dark (23L:1D) with a light intensity of 20 lux. Experimental lighting programs were 14L:10D, 17L:7D, 20L:4D and 23L:1D, with light provided by incandescent bulbs at a light intensity of 10 lux. Darkness was provided in one section per day. Lighting programs remained in place until the end of the experiments (31 or 32 d of age (d32), 38 or 39 d of age (d38) or 48 or 49 d of age (d48)).

Experiment one. In each of four trials, a total of eight rooms were randomly allocated to one of the four lighting programs described above, allowing two replications per lighting program per trial. Within each of the rooms, genotypes (Ross x Ross 308 and Ross x Ross 708) and bird genders were housed in small pens (8 or 12 per room). Each genotype by bird gender subclass was replicated two or three times per room. The total number of birds used in this work was 17,128.

Wheat straw was used as bedding and was placed within the pens to a depth of approximately 7.5 to 10 cm. Trials 1 and 2 were performed in a clean litter environment, where the barn was emptied of previous litter, washed, and a disinfectant used prior to new straw being placed. Trial 3 was performed with chicks being placed on the litter from

the previous trial (second flock on litter or re-used one time), while trial 4 included litter re-used two times (3rd flock on litter).

Experiment two. This experiment involved replication of the experiment 1 daylength treatments in two rooms with each room housing 944 mixed-sex Ross x Ross 308 broilers (equal number of males and females) in one large pen. The total number of birds used for this work was 7,552. This experiment was performed in a clean, fresh litter environment.

Data collection

Mortality and culls. Data were collected from experiment one (4 trials) to assess the impact of daylength, gender and genotype (308 and 708) on cause of morbidity and mortality. The second experiment examined the impact of daylength alone. Birds were monitored by barn staff twice per day, and birds that appeared ill (physical or behavioural symptoms) were culled. Although the decision for culling an ill bird was subjective, the primary reasons for culling were inability to move and loss of body weight. Birds were euthanized by cervical dislocation by trained staff members. All mortality and culled birds were necropsied for cause of death or morbidity by an independent laboratory (Prairie Diagnostics Services, Western College of Veterinary Medicine, University of Saskatchewan). The data were then categorized into one of five groupings based on etiology: infectious disorders (arthritis, polyserositis, peritonitis, osteomyelitis), metabolic disorders (Sudden Death Syndrome, ascites), skeletal disorders (valgus-varus, tibial dyschondroplasia, rotated tibia, spondylolithesis), unknown causes, and a final other category, in which individual numbers were minor (pendulous crop, twisted gastrointestinal tract, accidental deaths).

Gait score (GS). Bird mobility was assessed subjectively by the same two observers using the technique published by Garner et al. (2002). Within experiment 1, data were collected in trial 1 at 35 d of age, trial 2 at 35 and 47 d, and trial 4 at 28 and 45 d; each time, 40 birds per lighting treatment x gender x genotype were tested. In experiment 2, birds were scored at 37 d of age, and 60 birds per lighting treatment x gender were tested. Each time, individual birds were randomly selected, separated from their pen-mates, walked down a straw-covered pathway and scored by two GS testers. Once a consensus was reached by the GS testers, the score was recorded. The technique consists of a six point system, where 0 represents no abnormality in gait, and a 5 represents a complete loss of mobility. The data were then expressed as an average GS and as a percentage falling within each category. Based on work by Danbury et al. (2000), in which it was determined that pain was felt in the upper GS categories of 3 and over, the percentages falling in these categories were combined.

Footpad dermatitis. The same birds tested for GS were also examined for footpad dermatitis using the technique of Ekstrand et al. (1998). The footpad of the left foot was examined. A score of 0 represents a healthy footpad with no demarcation, while a score of 2 represents a severe lesion. The data were expressed as an average footpad score and as a percentage falling within each score.

Eye size. Eye weight was obtained from a sample of birds within experiment 1 (trials 3 and 4). Ten birds per lighting program x gender x genotype at each collection were weighed and then sacrificed via cervical dislocation. The eyes were removed, adhering tissue was trimmed and the two eyes were then weighed. The data were expressed as a total weight (g) and as a percentage of live weight.

Statistical analyses

All percentage data were transformed to (log+1) prior to analysis. As graded levels of light were chosen specifically to study relationships between the variables measured and daylength, the primary statistical technique used in both experiments was regression analysis (PROC REG (Regression) and PROC RSREG (Response Surface Regression)) of SAS. Differences were considered significant when the probability of difference was less than or equal to 0.05.

In experiment 1, the main effects of daylength, sex and genotype, as well as interactions between sex, genotype and lighting, were also analyzed using PROC GLM (General Linear Model) of SAS (SAS® 9.1., Cary, NC) as a 2 x 2 factorial nested within four lighting programs. When specific data were measured at the same age in more than one trial, trial was also used as a block within the model. Experiment 2 was analyzed as a one-way analysis of variance, with lighting program used as the main effect. When sexes were discernable (gait score or footpad score) a factorial model was used, with four lighting programs and two genders. When the ANOVA demonstrated significant differences ($P < 0.05$), then Duncan's Multiple Range Test was used to separate means.

RESULTS

Causes of mortality and morbidity

Daylength had significant linear or quadratic (depending on age) effects on many of the causes of mortality and culls (Table 1). Over the 7-32 d period, a positive linear relationship with daylength was noted for total mortality ($P=0.0006$) and for metabolic diseases ($P=0.0084$) and skeletal abnormalities ($P=0.0238$). Extending the grow-out period (7-38 d) resulted in a similar positive linear response for total mortality ($P=0.0026$) as well as metabolic ($P=0.0002$), skeletal ($P=0.0041$) and other ($P=0.0089$) disease conditions. When the growth period was extended even further (7-48 d), daylength

impacted all etiologies with the exception of the “other” category. Total mortality ($P=0.0008$) and metabolic ($P=0.0242$) and skeletal ($P=0.0065$) disease categories were once again influenced in a linear fashion, with the highest mortality found in birds exposed to the longest daylength. Quadratic responses were noted for infectious mortality (highest level occurring under 20L, $P=0.0315$) and for unknown causes ($P=0.0020$), where the highest level was noted under 14L followed by 23L, suggesting the relationship was not strong or predictive.

Table 2 shows the causes of mortality in Experiment 2. With fewer replications (two rooms per lighting program in one trial only), no significant relationships were found between lighting program and all causes.

Gait score

Gait scoring was used to assess bird mobility. The summarized data are shown in Table 3, and a strong similarity was noted at all ages. The percentage of birds falling in 0 category follow a linear (28 d, $P=0.0001$; 45 d, $P=0.0002$) or quadratic (35 d, $P=0.0030$) trend regardless of age measured, with the highest percentage of birds with no noted abnormality (scoring 0) found under shorter daylengths. Relationships were also linear or quadratic in the 1, 2 and 3 categories with the exception of 45 d birds in category 1, with the highest percentage occurring under long daylengths. The percent of birds falling into the upper GS categories of 3+4+5 were also impacted in a linear (28 d, $P=0.0301$; 35 d, $P=0.0001$; 45 d, $P=0.0005$) fashion regardless of age, with the poorest mobility noted under 23L. Average gait score was also impacted with either linear (28 d and 45 d, $P=0.0001$) or quadratic (35 d, $P=0.0009$) relationships.

Significance was more difficult to achieve in Experiment 2 (Table 4). Although numerical trends in all GS categories followed similar trends to those noted in experiment 1, only the percentage of birds falling into GS 0 responded in a nearly significant linear fashion ($P=0.0553$), with the highest percentage of normal birds (scoring 0) found under 14L.

Footpad lesion scores (FPLS)

The percentage of birds with FPLS of 0, 1, and 2 are shown in Table 5 along with average score. When measured at 28 d ($P=0.0374$) and 35 d ($P=0.0409$) of age, the average footpad score reacted linearly with daylength, with longer daylengths resulting in higher average FPLS. There were more birds falling under the 0 score (no lesions) under 14L at 35 d of age ($P=0.0494$), and the flocks reacted linearly with daylength. No relationships were noted at 45 d.

Ocular weight

The relationship between eye weight and daylength is shown in Table 6. At 31 ($P=0.0001$) and 46 d ($P=0.0033$), the relationship was identical, with eye weight increasing in a quadratic fashion with daylength. Closer examination of the data indicates that values are similar under 14, 17 and 20L, but that eye weight under 23L is significantly heavier.

Gender and genotype

Differences in the causes of mortality were impacted by gender and genotype (Table 7). Regardless of the period examined, males were more susceptible to death from metabolic and skeletal diseases and had higher mortality overall than females. Females in the 7-32 d and 7-48 d period had a higher level of mortality from diseases included in the “other” category than males. Overall mortality due to infection for all age

groups were higher for the 308 than the 708 genotype. The proportion of birds classified as unknown (7-32 d, 7-38 d), other (7-32 d) and metabolic disease (7-48 d) mortality were also higher for the 308 genotype. Only one interaction between strain and sex was noted, and only in one period (7-48 d period), where more male 708 broilers died from diseases in the other category causes than their male 308 counterparts (0.99% vs. 0%). The reverse was seen for females (708, 0.09%; 308, 0.59%). The incidence in all cases was less than 1%, so this may have been a chance occurrence rather than a biological effect.

Gender resulted in differences in mobility, and in all cases in experiment 1 females were more mobile than males (Table 8). Mobility was impacted by genotype sporadically, but the differences were not evident for all ages (Table 8). Interactions in experiment 1 were inconsistent. At 28 d of age, the percent of birds falling into category 4 and those into 3+4+5 demonstrated a light x gender interaction. No females or males raised under 14 or 17L scored in the 4 category, but 1.56% were found under 20L and 4.69% under 23L. Similarly, daylength resulted in increasing numbers of males in the 3+4+5 category (0, 1.56, 4.69 and 9.38% for 14, 17, 20 and 23L, respectively), while little impact was seen in the females (0, 3.13, 0 and 0% for 14, 17, 20 and 23L, respectively). At 35 d, genotypes reacted differently to lighting programs. In both cases, the significance results from a difference in magnitude rather than a change in ranking. The frequency of birds classified in GS 2 were higher under long daylengths, but the increase in the 708 birds was higher (308's: 1.88, 1.88, 1.88 and 3.33% for 14, 17, 20 and 23L, respectively; 708's: 0.63, 1.25, 3.13 and 8.00% for 14, 17, 20 and 23L, respectively). Again, higher percentages falling in the 3+4+5 category were noted in the long daylength groups but, in this case, a higher level was found for the 308 (0, 0.63, 3.75 and 7.33% for 14, 17, 20 and 23L, respectively) in comparison to the 708 broilers (0, 0.63, 1.25, and 1.33% for 14, 17,

20 and 23L, respectively). Finally, at 42 d the average GS indicated that the sexes reacted differently within genotypes. The average gait score was higher for male 708 than for male 308 broilers (12.83 and 14.19% for 308 and 708s, respectively), but in the females the ranking was opposite (6.41 and 5.30% for 308 and 708s, respectively).

The effect of gender and genotype on the incidence of foot pad lesions is shown in Table 10. At 28 and 35 d of age, males had a lower mean score than did females, indicating less severe lesions. While the trend was similar at 45 d, no significance was noted. Genotype differences were noted at all ages, with higher values for 308 than 708 broilers. At 28 d of age, an interaction was noted between sex and daylength. The percentage of male birds falling into the most severe FPLS of 2 increased in a linear fashion with daylength (1.56, 3.13, 6.25 and 12.50% for 14, 17, 20 and 23L, respectively). Females did not show a consistent effect due to lighting program, and the highest percentage was found under 17L (4.52, 9.38, 4.69 and 4.92% for 14, 17, 20 and 23L, respectively).

Males had heavier eyes than females at both ages, but when corrected for body weight there was no effect (Table 11). Percentage eye weight was higher for 708 than 308 birds, but absolute weights did not differ. A significant interaction between daylength and genotype was noted for the 31 day proportional data. The data show that eye weight is similar for the two genotypes under 14L, 17L, and 20L, but that the 708 birds had heavier eyes under 23L (308: 0.20, 0.18, 0.18 and 0.21 for 14, 17, 20 and 23L, respectively; 708: 0.20, 0.20, 0.20 and 0.25% for 14, 17, 20 and 23L, respectively). An interaction was also found for light x genotype in absolute eye weight at 46 d of age but a lack of clear trend in the data suggest that this is due to chance variation.

Discussion

Poor health is likely the most obvious signal of welfare (Dawkins et al., 2004). While there have been many studies in the past comparing the impact of lighting programs on health status in broiler flocks, few have attempted to understand how much darkness is required to maximize health in a flock. Two such works were conducted by Gordon (1997) and Lewis et al. (2009c). Gordon (1997) discussed the relationship between mortality and daylength, but neither materials and methods nor actual data were included in the literature review, and therefore critical review is not possible. The report included a comparison of birds exposed to 8, 12, 16 or 20 h of light; birds responded to liveability in a curvilinear manner, with long dark periods having the lowest mortality. Lewis et al. (2009c), using one lighting treatment room replication (200 broilers per genotype per room) compared a range of daylengths including 2, 4, 6, 8, 10 (in trial 1 only), 12, 15, 18, 21 and 24 h in each of two trials. The results indicated that mortality increased with photoperiod length above 12 h, but no differences below 12 h of light per day. Relatively small bird numbers per group and low replication may have made differences difficult to find in measurements that typically have high variability between replicates.

It is apparent from the current study that daylength does have an important impact on the health parameters measured, which in turn alters broiler welfare. The use of graded daylength treatments and appropriate replication in this work adds definition to interpretation of these responses. In this work, the provision of darkness to broiler chickens improved overall liveability in a linear fashion. The largest impact was on incidence of metabolic disease ((sudden death syndrome) and chronic (ascites) heart failure, with the former of predominant importance) and on skeletal health, which is in agreement with previous research (Robbins et al., 1984; Classen and Riddell, 1989;

Classen et al., 1991). Daylength also affected incidence of infectious disease in older birds and this may relate to the beneficial effects of darkness on immune function (Kliger et al., 2000). Other daylength effects on unknown and other categories add little to the understanding of darkness benefits because of their low incidence and/or undefined nature.

Daylength impacted broiler mobility, with ability to move decreasing with increasing daylength. A reduced ability to move as defined by gait score can indicate a pathological or physiological condition that, depending on severity, can be a welfare concern. Danbury et al. (2000) fed analgesics to lame broilers in an attempt to determine if lameness caused pain, and found that birds in the gait score categories of 3 or higher were in pain, constituting a welfare concern. Regardless of the age measured, a positive linear relationship existed between daylength and the percentage of birds in categories above 2. These results substantiate previous research, which has indicated that shorter daylength (increased darkness) reduces the incidence of leg weakness (Classen and Riddell, 1989; Sanotra et al., 2002). This finding is in close agreement with the response of observed mortality due to skeletal disease to daylength. The similarity of these trends suggests that gait scoring techniques, despite their subjective nature, accurately define leg weakness in broilers.

Rapid growth rate associated with long daylengths is often implicated as the cause of increased mortality, particularly with regards to metabolic and skeletal disease. Data from the present research show that growth rate is not the only factor involved. Birds raised on 20L:4D were the heaviest at 32 d of age (Schwean-Lardner et al., 2011), yet mortality was higher and mobility poorer in birds raised under 23L:1D. This indicates that lack of darkness itself impacts these health associated factors rather than absolute growth rate always dictating the incidence levels and reducing welfare. At older ages,

when birds appeared to better adapt to longer dark periods, market body weights of birds exposed to 7 or even 10 h of darkness were as heavy or heavier than those of birds raised under 23L:1D; however, mortality in every case again followed a linear relationship with daylength, with the highest rate occurring under near-continuous light and mobility being poorer under long daylengths. These data support the concept that the timing of rapid growth is important, with less detrimental impacts if rapid growth occurs later in life after a strong skeletal foundation has been established during early life. The shifts in growth patterns associated with day length have been described previously (Schwean-Lardner et al., 2011).

Exercise is often cited as a reason why shorter daylength and longer dark periods affect incidence of broiler leg weakness and associated mortality. Data from an associated study (Schwean-Lardner et al., 2006b) utilized scan-sampling techniques every 10 minutes over a 24-h period to accurately define behavioural differences associated with graded hours of daylength. Exposure to darkness increased exercise (walking and standing) linearly when measured at 27-28 d, with little activity occurring under near-constant light. As birds aged, long daylengths still resulted in a reduction in exercise, but a quadratic relationship determined that behaviour under 7 and 10 h of darkness was similar. In all cases, 20 h of light produced intermediate results. Other behaviours followed similar trends, including time at the feeder, preening, dustbathing and stretching.

In this work, the response pattern of mortality and incidence of leg weaknesses are similar to that seen in behaviour, with mortality due to skeletal disease and percent of birds falling in GS categories of 3+4+5 increasing linearly with daylength over all time periods in experiment 1. Common trends observed support an exercise-based mechanism for improved health in broilers given increased darkness exposure. However,

it is not clear how much exercise is required to reduce leg weakness, and an increase in total activity (exercise) over a 24-h period of time with shorter daylength does not prove a cause and effect relationship. It is known that exercise can improve bone health (Goktepe et al., 2008), but research on the impact of exercise on mortality and leg weakness in poultry has been conflicting. As an example, Balog et al. (1997) used ramps and/or toys to increase exercise in two separate trials. Cumulative mortality was reduced by ramps leading to feeders in their second trial, but no impact was found in the first trial. In apparent contradiction, bone breaking strength was increased in birds with ramps in trial 1 but not in trial 2, further demonstrating the inconsistency in results. Because skeletal health has a multifactorial basis, it is probable that exercise is only one of several factors capable of improving health and reducing leg weakness as a result of darkness exposure in broiler chickens.

Near-constant light caused birds to have heavier eyes than did birds on any other lighting program. The eye grows in a circadian fashion in response to fluctuation in melatonin production, with growth during the light period and cessation during the dark (Rada and Wiechmann, 2006). When exposed to long periods of daylength, this rhythm is disturbed and consequently results in heavier and distorted eyes (Li et al., 1995). Our results are in agreement with research using turkey poults where exposure to both 24L:0D or 23L:1D resulted in heavier eyes than did exposure to photoperiods that included substantial dark periods. An accumulation of fluid in the eye results in the heavier weights, and could possibly increase intraocular pressure (Smith et al., 1969). Long-term changes in eye weight can lead to thickening of the cornea, damage to the retina and other components of the eye, and cataract development (Li et al., 1995). The potential for reduced vision and pain from increased ocular pressure would reduce bird welfare. A lack of a melatonin rhythm when birds are exposed to near-continuous light

(Schwean-Lardner et al., 2010) supports the potential for increased eye weight of birds not given darkness exposure because of the disruption of eye growth rhythms. Our finding that birds given 4, 7 or 10 h darkness had identical eye weights suggests that as little as 4 h of darkness is enough to trigger the circadian eye growth pattern.

Footpad dermatitis is a skin lesion that occurs on the bottom of the foot. These lesions can result in bird discomfort and pain (Bradshaw et al., 2002) and are a welfare consideration. Research has shown that lighting programs have an inconsistent impact on the development of these lesions. Petek et al. (2010) compared birds on intermittent light to those on continuous light and found no differences. Sørensen et al. (1999) compared birds raised on 8 h of light to 16 h in one trial, and 16 h to 21 h in another trial, and found footpad lesions to be less severe on long photoperiods when corrected for body weight. In contrast, a linear increase in average lesion severity was seen with increasing daylengths in the current research.

Footpad dermatitis is impacted by a variety of management factors, and light may only play a small role. Activity, for example, may improve litter quality as a result of more frequent turn-over, which in turn reduces lesions (Kristensen et al., 2004). There may also be less continuous contact of skin with litter when activity levels are higher, again resulting in a reduction in lesion severity. While activity levels were not reported in the other research mentioned, another segment of this work (Schwean-Lardner et al., 2006b) did show linear declines in activity with increasing daylight. Studies in rats suggest that skin integrity, specifically thickness, is impacted by melatonin production (Eşrefoğlu et al., 2005). If this is the case in poultry, litter irritation would cause more severe burns in thin-skinned birds as compared to those with thicker skin as a result of increased melatonin secretion. To the authors' knowledge, this association has not been made in poultry. Of interest, Schwean-Lardner et al. (2010) showed that the expected pattern of melatonin

production is eliminated by long daylengths. Therefore, it could be that a combination of litter quality and skin integrity effects due to daylength in this work resulted in the noted impact on lesions.

Sleep deprivation can have a large impact on health. For example, sleep deprivation in rats as a result of repeated awakenings caused a variety of physical problems, including muscular weakness, foot pad lesions, a reduction in brain function, and a wide range of pathological diseases found on necropsy. In some cases, sleep deprivation led to death (Rechtschaffen et al., 1983). Evidence exists to link melatonin production and sleep deprivation, and a lack of a melatonin rhythm can suppress sleep (Yamada et al., 1988); hence, the two are likely intertwined. Therefore, the impact on sleep and its patterns is a concern when discussing lighting programs (Blokhus, 1983), particularly in relationship to day and night rhythms. The degree of darkness required to provide adequate sleep is difficult to determine. Results from the present study suggest that birds exposed to 20L are achieving some degree of sleep, but not enough. This is indicated by only intermediate behavioural expression (Schwean-Lardner et al., 2006b), a shorter melatonin rhythm (Schwean-Lardner et al., 2010), and poorer health for birds exposed to 4 h darkness as compared to those with longer dark periods.

Gender

Males had a higher incidence of mortality and were less mobile than females in this study. This is in agreement with previous research and is likely related to the growth associated higher demands on skeletal (Sørensen et al., 1999) and metabolic systems (Peacock et al., 1990) in males than females. This is supported by an associated study that found that males grew faster and were heavier than females (Schwean-Lardner et al., 2011). Daylength impacted the causes of death in both sexes similarly. Males exhibited poorer mobility in general and had a higher percentage of birds falling in upper

GS categories in experiment 1 at 28 d. Males have stronger skin than females (Kafri et al., 1986), and this could be reflected in less severe average footpad lesions.

Genotype

The two genotypes used in this work are quite diverse, with the 308 genotype growing quickly in contrast to the 708, which grows more slowly and has greater breast meat deposition. The faster growing 308 birds had either significantly or numerically higher mortality levels overall, attributed to metabolic and infectious causes particularly over the 7-48 d period. It is important to note that both genotypes responded to the changes in daylength in a similar manner. Average gait scores under 1 for both strains at all time periods indicate good leg health, and may be reflective of selection pressures placed on the genotypes by the Primary Breeder Company.

In conclusion, daylength has a significant impact on many aspects of broiler health. By reducing the daylength, the economics of broiler production can be improved, as mobile birds are better able to reach feeders and waterers, more birds are marketed, and condemnations and downgrading are reduced. Daylength also likely affected pain due to leg weakness and liveability, with more darkness improving these traits. Therefore, the use of lighting programs with significant darkness will improve broiler welfare.

Although the welfare implications are not fully understood, larger eye weight in birds raised under near-constant light is a concern. Welfare implications include the physical aspect of eye enlargement with potential detrimental effects as well as a lack of circadian rhythms. The data in the current study indicate that constant or near-constant lighting programs should not be used for broiler production. Although statistics show a linear nature in the response of the data in this work, it is interesting to note that mortality is similar or even higher under 14L than 17L, and this suggests that an appropriate length of

darkness for maximizing broiler welfare based on health parameters alone approximates 7 h per day.

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TABLE 3.1. Effect of daylength on cause of mortality and culls (% of birds placed) from light program initiation to 32, 38 and 48 days of age. – Experiment 1

	Daylength (h)				SEM ¹	P value ²	Equation
	14	17	20	23			
<u>7-32 d</u>							
Total	2.30	2.34	3.10	3.59	0.143	0.0006	Y=-0.06395+0.15672x
Metabolic ³	1.23	1.20	1.82	1.73	0.106	0.0084	Y=0.15630+0.072507x
Skeletal ⁴	0.38	0.25	0.50	0.71	0.060	0.0238	Y=0.35240+0.10970x
Infectious ⁵	0.48	0.59	0.62	0.63	0.066	NS	-
Unknown cause	0.14	0.23	0.08	0.39	0.035	NS	-
Other ⁶	0.07	0.07	0.08	0.13	0.024	NS	-
<u>7-38 d</u>							
Total	3.03	2.97	3.98	5.04	0.218	0.0026	Y=-0.59077+0.23493x
Metabolic	1.42	1.35	2.00	2.36	0.148	0.0002	Y=-0.35999+0.11591x
Skeletal	0.77	0.40	0.86	1.11	0.105	0.0041	Y=-0.12492+0.04914x
Infectious	0.67	0.83	0.91	0.87	0.097	NS	-
Unknown cause	0.09	0.35	0.12	0.58	0.052	NS	-
Other	0.07	0.04	0.09	0.12	0.029	0.0089	Y=-0.03708+0.00646x

TABLE 3.1. Effect of daylength on cause of mortality and culls (% of birds placed) from light program initiation to 32, 38 and 48 days of age. – Experiment 1

	Daylength (h)				SEM ¹	P value ²	Equation
	14	17	20	23			
<u>7-48 d</u>							
Total	3.64	3.88	7.13	6.56	0.424	0.0008	Y=-0.3591+0.1308x
Metabolic	1.91	1.54	2.96	2.99	0.286	0.0242	Y=-0.3300+0.0712x
Skeletal	0.36	0.73	1.71	1.48	0.196	0.0065	Y=-0.6862+0.0635x
Infectious	0.65	1.44	2.01	1.30	0.185	0.0315	Y=-13.9681+1.6223x-0.0416x ²
Unknown cause	0.63	0	0.18	0.54	0.081	0.0020	Y=-9.4673-1.0164x+0.0274x ²
Other	0.09	0.17	0.27	0.26	0.069	NS	-

Values listed for lighting program means and for standard error of means (SEM) and regression equation calculation based on original data.

¹ SEM – Standard error of the mean.

² Values for *P* (probability of regression) based on log transformed values.

³ Metabolic disease – sudden death syndrome, ascites.

⁴ Skeletal – valgus-varus, tibial dyschondroplasia, rotated tibia, spondylolithesis.

⁵ Infectious – arthritis, polyserositis, peritonitis, osteomyelitis.

⁶ Other – pendulous crop, twisted gastrointestinal tract, accidental death.

TABLE 3.2. Effect of daylength on cause of mortality and culls (% of birds placed) from light program initiation to 32, and 38 days of age – Experiment 2

	Daylength (h)				SEM ¹	<i>P</i> value ²	Equation
	14	17	20	23			
<u>7-32 d</u>							
Total	2.28	3.07	2.33	2.86	0.244	NS	-
Metabolic ³	1.37	1.91	1.27	1.85	0.157	NS	-
Skeletal ⁴	0.16	0.11	0	0.37	0.072	NS	-
Infectious ⁵	0.42	0.69	0.64	0.37	0.100	NS	-
Unknown cause	0.21	0.26	0.26	0.11	0.060	NS	-
Other ⁶	0.11	0.11	0.16	0.16	0.039	NS	-
<u>7-38 d</u>							
Total	2.64	3.76	3.07	3.87	0.266	NS	-
Metabolic	1.54	2.11	1.64	2.44	0.197	NS	-
Skeletal	0.16	0.21	0.11	0.48	0.066	NS	-
Infectious	0.64	0.85	0.74	0.58	0.128	NS	-
Unknown cause	0.21	0.42	0.42	0.11	0.082	NS	-
Other	0.11	0.16	0.16	0.26	0.056	NS	-

Values listed for lighting program means and for standard error of means (SEM) and regression equation calculation based on original data.

TABLE 3.2. Effect of daylength on cause of mortality and culls (% of birds placed) from light program initiation to 32, and 38 days of age – Experiment 2

Daylength (h)				SEM ¹	P value ²	Equation
14	17	20	23			

¹ SEM – Standard error of the mean.

² Values for the P (probability of regression) based on log transformed values.

³ Metabolic disease – sudden death syndrome, ascites.

⁴ Skeletal – valgus-varus, tibial dyschondroplasia, rotated tibia, spondylolithesis.

⁵ Infectious – arthritis, polyserositis, peritonitis, osteomyelitis.

⁶ Other – pendulous crop, twisted gastrointestinal tract, accidental death.

TABLE 3.3. The effect of daylength on the percentage of birds falling in gait score categories (as described by Garner et al., 2002), the total of categories 3+4+5 and the mean gait score at 28, 35 and 45 d of age – Experiment 1

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>28 d</u>							
0	47.78	32.81	23.44	15.63	2.156	0.0001	Y=95.1823-3.5280x
1	42.84	55.47	63.28	60.04	2.381	0.0044	Y=18.7723+1.9804x
2	9.38	9.38	10.94	19.64	1.538	0.0174	Y=-7.6265+1.0788x
3	0	2.34	1.56	2.34	0.653	0.0207	Y=-4.0365+0.2604x
4	0	0	0.78	2.34	0.381	NS	-
5	0	0	0	0	0	NS	-
3+4+5	0.000	2.34	2.34	4.69	0.730	0.0301	Y=-6.3281+0.4688x
Mean	0.62	0.81	0.93	1.16	0.041	0.0001	Y=-0.1950+0.0581x
<u>35 d</u>							
0	72.81	70.00	67.50	45.33	1.836	0.0030	Y=-2.8225+0.7845x-0.0247x ²
1	25.94	27.81	27.50	44.67	1.742	0.0214	Y=6.3285-0.6366x+0.0195x ²
2	1.25	1.56	2.50	5.67	0.457	0.0005	Y=-5.9212+0.4677x
3	0	0.63	2.50	3.67	0.386	0.0002	Y=-6.2378+0.4289x
4	0	0	0	0.67	0.112	NS	-

5	0	0	0	0	0	NS	-
3+4+5	0	0.63	2.50	4.33	0.413	0.0001	Y=-7.2835+0.1161x
Mean	0.28	0.33	0.40	0.70	0.023	0.0009	Y=1.9407-0.2157x+0.0070x ²
<u>45 d</u>							
0	28.82	22.05	10.40	11.51	1.945	0.0002	Y=57.4758-2.1260x
1	49.77	49.28	50.64	40.76	1.835	NS	-
2	16.75	19.42	30.25	31.47	2.221	0.0051	Y=-9.4864+1.8373x
3	2.81	7.20	7.87	15.13	1.006	0.0001	Y=-14.9131+1.2509x
4	0.46	1.60	0.40	0.67	0.356	NS	-
5	1.39	0.46	0.44	0.46	0.273	NS	-
3+4+5	4.66	9.26	8.72	16.26	1.117	0.0005	Y=-11.3544+1.1379x
Mean	1.00	1.18	1.39	1.54	0.047	0.0001	Y=0.1630+0.0603x

Values listed for lighting program means and for standard error of means (SEM) and regression equation calculation based on original data.

¹ SEM – Standard error of the mean.

² Values for *P* (probability of regression) and *R*² based on log-transformed values.

TABLE 3.4. The effect of daylength on the percentage of birds falling in gait score categories (as described by Garner et al., 2002), the total of categories 3+4+5 and the mean gait score at 36 d of age. – Experiment 2

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>36 d</u>							
0	46.88	37.50	35.94	28.13	3.422	0.0553	Y=72.7604-1.9271x
1	46.88	56.25	57.03	60.94	2.849	NS	-
2	2.34	2.34	4.69	4.69	0.748	NS	-
3	3.91	3.13	2.34	5.47	0.866	NS	-
4	0	0	0	0.78	0.195	NS	-
5	0	0.78	0	0	0.195	NS	-
3+4+5	3.91	3.91	2.34	6.25	0.934	NS	-
Mean	0.63	0.74	0.73	0.90	0.051	NS	-

Values listed for lighting program means and for standard error of means (SEM) and regression equation calculation based on original data.

¹ SEM – Standard error of the mean.

² Values for *P* (probability of regression) and *R*² based on log-transformed values.

TABLE 3.5. The effect of daylength on the percentage of birds falling in footpad lesion score categories of 0, 1, or 2 (as described by Ekstrand et al., 1998) and the mean footpad lesion score at 28, 35 and 45 d of age – Experiment 1

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>28 d</u>							
0	75.26	70.31	62.50	60.60	3.020	NS	-
1	21.70	23.44	32.03	30.69	2.630	NS	-
2	3.04	6.25	5.47	8.71	1.042	NS	-
Mean	0.28	0.36	0.43	0.48	0.036	0.0374	Y=-0.0342+0.02267x
<u>35 d</u>							
0	52.50	50.94	40.94	44.67	1.924	0.0494	Y=68.1730-1.1326x
1	47.50	48.13	58.44	54.33	1.880	NS	-
2	0	0.94	0.63	1.00	0.245	NS	-
Mean	0.48	0.50	0.60	0.56	0.020	0.0409	Y=0.3081+0.0122x

TABLE 3.5. The effect of daylength on the percentage of birds falling in footpad lesion score categories of 0, 1, or 2 (as described by Ekstrand et al., 1998) and the mean footpad lesion score at 28, 35 and 45 d of age – Experiment 1

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>45 d</u>							
0	64.41	64.55	62.22	65.02	2.789	NS	-
1	22.74	19.20	19.35	18.49	1.843	NS	-
2	12.85	16.25	18.43	16.49	1.956	NS	-
Mean	0.48	0.52	0.56	0.51	0.045	NS	-

Values listed for lighting program means and for standard error of means (SEM) and regression equation calculation based on original data.

¹ SEM – Standard error of the mean.

² Values for *P* (probability of regression) and *R*² based on log-transformed values.

TABLE 3.6. The effect of daylength on the absolute and relative ocular weight as compared to live weight at 31 and 46 d of age – Experiment 1

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>31 d</u>							
Absolute (g)	4.02 ^B	3.87 ^B	3.99 ^B	4.66 ^A	0.048	0.0001	Y=-0.775x+0.023x ² +10.423
Relative (g/kg body wt)	0.20 ^B	0.19 ^B	0.19 ^B	0.23 ^A	0.002	0.0001	Y=-0.535+0.042x+0.001x ²
<u>46 d</u>							
Absolute (g)	4.80	4.85	4.77	5.39	0.050	0.0033	Y=4.777+0.253x+0.315x ²
Relative (g/kg body wt)	0.154	0.159	0.157	0.178	0.002	0.0039	Y=0.278-0.016x+0.0005x ²

For percent of live data, values listed for lighting program means and for standard error of means (SEM) and regression equation

¹ SEM – Standard error of the mean.

² Values for *P* (probability of regression) and *R*² based on log-transformed values for percent data only.

TABLE 3.7. Effect of gender and genotype on cause of mortality and culls (% of birds placed) from light program initiation to 32, 38 and 48 days of age – Experiment 1

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>7-32 d</u>								
Metabolic ¹	2.30 ^A	0.70 ^B	1.63	1.36	NS	NS	NS	NS
Skeletal ²	0.65 ^A	0.27 ^B	0.48	0.44	NS	NS	NS	NS
Infectious ³	0.56	0.59	0.77 ^a	0.38 ^b	NS	NS	NS	NS
Unknown	0.16	0.26	0.29 ^a	0.13 ^b	NS	NS	NS	NS
Other ⁴	0.04 ^B	0.14 ^A	0.15 ^a	0.03 ^b	NS	NS	NS	NS
Total	3.71 ^A	1.96 ^B	3.33 ^a	2.34 ^b	NS	NS	NS	NS
<u>7-38 d</u>								
Metabolic	2.82 ^A	0.75 ^B	1.81	1.76	NS	NS	NS	NS
Skeletal	1.07 ^A	0.50 ^B	0.77	0.80	NS	NS	NS	NS
Infectious	1.02 ^A	0.62 ^B	1.00	0.64	NS	NS	NS	NS
Unknown	0.23	0.34	0.39	0.18	NS	NS	NS	NS
Other	0.05	0.12	0.10 ^a	0.07 ^b	NS	NS	NS	NS
Total	5.18 ^A	2.33 ^B	4.06	3.45	NS	NS	NS	NS

TABLE 3.7. Effect of gender and genotype on cause of mortality and culls (% of birds placed) from light program initiation to 32, 38 and 48 days of age – Experiment 1

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>7-48 d</u>								
Metabolic	3.64 ^A	1.05 ^B	3.14 ^a	1.55 ^b	NS	NS	NS	NS
Skeletal	1.47 ^A	0.67 ^B	1.11	1.03	NS	NS	NS	NS
Infectious	1.43	1.27	2.88 ^a	0.82 ^b	NS	NS	NS	NS
Unknown	0.30	0.38	0.36	0.32	NS	NS	NS	NS
Other	0.05 ^B	0.34 ^A	0.30	0.09	NS	NS	0.0284	NS
Total	6.89 ^A	3.71 ^B	6.80 ^a	3.81 ^b	NS	NS	NS	NS

^{A, B} Means with common letters do not differ significantly ($P < 0.05$).

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

¹ Metabolic disease – sudden death syndrome, ascites.

² Skeletal – valgus-varus, tibial dyschondroplasia, rotated tibia, spondylolithesis.

³ Infectious – arthritis, polyserositis, peritonitis, osteomyelitis.

⁴ Other – pendulous crop, twisted gastrointestinal tract, accidental death.

TABLE 3.8. Effect of gender and genotype on birds falling in gait score categories of 0, 1, 2, 3, 4 and 5, the combination of 3, 4 and 5 and mean gait score at 28, 35 and 45 d of age– Experiment 1

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>28 d</u>								
0	25.00 ^B	34.83 ^A	33.48	26.35	NS	NS	NS	NS
1	55.08	55.74	55.97	54.85	NS	NS	NS	NS
2	16.02 ^A	8.65 ^B	9.77	14.90	NS	NS	NS	NS
3	2.34	0.78	0 ^b	3.13 ^a	NS	NS	NS	NS
4	1.56 ^A	0 ^B	0.78	0.78	0.0438	NS	NS	NS
5	0	0	0	0	NS	NS	NS	NS
3+4+5	3.91 ^A	0.78 ^B	0.78 ^b	3.91 ^a	0.0094	NS	NS	NS
Mean	1.00 ^A	0.75 ^B	0.79 ^b	0.97 ^a	NS	NS	NS	NS
<u>35 d</u>								
0	60.31 ^B	68.23 ^A	62.86	65.56	NS	NS	NS	NS
1	33.75	28.71	32.06	30.48	NS	NS	NS	NS
2	3.59 ^A	1.77 ^B	2.22	3.17	NS	0.0362	NS	NS
3	2.03	1.29	2.54 ^a	0.79 ^b	NS	NS	NS	NS

4	0.31	0	0	0	NS	NS	NS	NS
5	0	0	0	0	NS	NS	NS	NS
3+4+5	2.34	1.29	2.86 ^a	0.79 ^b	NS	0.0150	NS	NS
Mean	0.48 ^A	0.36 ^B	0.45 ^a	0.39 ^b	NS	NS	NS	NS
<u>45 d</u>								
0	12.89 ^A	23.44 ^B	19.84	16.35	NS	NS	NS	NS
1	47.30	47.99	48.94	46.32	NS	NS	NS	NS
2	26.32	22.71	21.54 ^b	27.59 ^a	NS	NS	NS	NS
3	11.60 ^A	4.83 ^B	7.89	8.61	NS	NS	NS	NS
4	0.99	0.57	0.89	0.67	NS	NS	NS	NS
5	0.91	0.46	0.91	0.46	NS	NS	NS	NS
3+4+5	13.49 ^A	5.87 ^B	9.68	9.74	NS	NS	NS	NS
Mean	1.43 ^A	1.12 ^B	1.24	1.32	NS	NS	0.0159	NS

^{A, B} Means with common letters do not differ significantly ($P < 0.05$).

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

TABLE 3.9. Effect of gender on birds falling in gait score categories of 0, 1, 2, 3,4 and 5. the combination of 3, 4 and 5 and the mean gait score at 28 d of age – Experiment 2

	Gender		Light x Gender
	Male	Female	
<u>28 d</u>			
0	32.81	41.41	NS
1	55.86	54.69	NS
2	5.47 ^A	1.56 ^B	NS
3	5.47	1.95	NS
4	0	0.39	NS
5	0.39	0	NS
3+4+5	5.86	2.34	NS
Mean	0.85	0.65	NS

^{A, B} Means with common letters do not differ significantly (P<0.05).

TABLE 3.10. Effect of gender and genotype on birds falling in footpad score categories of 0, 1 and 2, and the mean footpad score at 38, 35 and 45 d of age.

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>28 d</u>								
0	72.66 ^A	61.68 ^B	55.86 ^b	78.48 ^a	NS	NS	NS	NS
1	21.48 ^B	32.45 ^A	34.77 ^a	19.17 ^b	NS	NS	NS	NS
2	5.86	5.87	9.38 ^a	2.36 ^b	0.0008	NS	NS	NS
Mean	0.33 ^B	0.44 ^A	0.54 ^a	0.24 ^b	NS	NS	NS	NS
<u>35 d</u>								
0	53.13 ^A	41.29 ^B	42.38 ^b	52.22 ^a	NS	NS	NS	NS
1	58.55 ^A	45.78 ^B	56.83 ^a	47.30 ^b	NS	NS	NS	NS
2	1.09 ^A	0.16 ^B	0.79	0.48	NS	NS	NS	NS
Mean	0.48 ^B	0.59 ^A	0.58 ^a	0.48 ^b	NS	NS	NS	NS

TABLE 3.10. Effect of gender and genotype on birds falling in footpad score categories of 0, 1 and 2, and the mean footpad score at 38, 35 and 45 d of age.

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>45 d</u>								
0	68.71	59.19	55.50 ^b	72.80 ^a	NS	NS	NS	NS
1	17.21	22.75	22.45	17.34	NS	NS	NS	NS
2	14.08	18.06	22.04 ^a	9.85 ^b	NS	NS	NS	NS
Mean	0.45	0.59	0.66 ^a	0.37 ^b	NS	NS	NS	NS

^{A, B} Means with common letters do not differ significantly (P<0.05).

^{a, b} Means with common letters do not differ significantly (P<0.05).

TABLE 3.11. Effect of gender and genotype on absolute ocular weight and the percentage of ocular weight as compared to live weight – Experiment 1.

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>31 d</u>								
Wt g	4.42 ^A	3.85 ^B	4.13	4.14	NS	NS	NS	NS
% of live	0.20	0.20	0.19 ^b	0.21 ^a	NS	0.0322	NS	NS
<u>46 d</u>								
Wt g	5.36 ^A	4.54 ^B	4.96	4.94	NS	0.0001	NS	NS
% of live	0.16	0.16	0.16 ^b	0.17 ^a	NS	NS	NS	NS

^{A, B} Means with common letters do not differ significantly ($P < 0.05$).

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

Preface to Chapter 4: The impact of daylength on behavioural output in commercial broilers

How an animal feels is very difficult to interpret, but the knowledge gained from it is vital in the establishment of level of welfare. The primary technique in understanding how an animal feels is behavioural observation, to look for signals of comfort, negative or positive behaviours. The focus of Chapter 4 was the examination of behavioural output of male Ross x Ross 308 broilers.

Chapter 4. The impact of daylength on behavioural output in commercial broilers*

* A version of this chapter has been submitted to Applied Animal Behaviour Science. Schwean-Lardner, K., B.I. Fancher, and H.L. Classen. Effect of daylength on behavioural output of broilers. August 2011

ABSTRACT Assessing broiler behaviour is one tool helpful in assessing welfare, including the impacts of management systems, such as lighting programs. This study examined broiler behaviour at two ages in two separate flocks (27-28 d of age in flock one and 42-43 d of age in flock two) as affected by one of four lighting programs (14L:10D (14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L)). The lighting programs were chosen so that relationships between the percentage of time spent on particular behaviours and daylength could be examined using regression analyses. Data were recorded with the use of an infrared camera system and digitalized to computer hard drive. Data were then observed using a scan-sample technique, and the number of birds performing particular behaviours was recorded every 10 minutes for the full 24-h period (6 measurements x 24 h per d) in two replicate pens per lighting treatment per age. The data, as a percentage of time, were tested for normality and (log+1) transformed prior to analysis. Regardless of age, photoperiod length impacted behaviour during the photoperiod and when averaged over a 24-h period. A negative linear or quadratic reduction in percent of time spent standing, walking, feeding, preening, stretching, dustbathing and foraging with increasing daylength was found at both ages in both the photoperiod and 24 h average. A similar reduction was found for running for the photoperiod and 24 h day at 27/28 d with the behaviour eliminated under 23L. Dustbathing was no longer present in the behavioural repertoire of the latter treatment birds at 42/43 d of age. Long photoperiods resulted in a reduced reactivity of birds to the presence of an observer, and in the sound level within a room of birds. Differences in percent time of behaviour were non-existent or minor in birds raised on 14L or 17L, and reactivity was similar at 27 d but higher in 14L birds at 42 d. Sound levels within these rooms were similar. Birds raised on 20 h of light were intermediate in behavioural expression. In conclusion, the decrease or elimination of behaviours, including those that are highly motivated or necessary for health, indicates poor welfare in birds raised on near-constant light. However, a long scotoperiod such as in the 14L:10D period does not appear to improve

welfare as monitored by behaviour. Optimal welfare, based on behaviour, can be attained with a photoperiod such as 17L.

Key words: photoperiod, light, dark, behaviour, sound

INTRODUCTION

Lighting programs are not always used in commercial poultry production systems, and the use of constant or near-constant light is common. Historically, producers have chosen to use this lighting regime for varied reasons, including allowing birds long visual access to feeders and waterers that hypothetically results in maximum feed intake and body weight (Morris, 1967; Savory, 1976; Classen, 1992; Buyse et al., 1996a; Lewis and Morris, 2006) and a reduction in activity, making for easier catching at market time. However, the first concept has been shown not to be true. Despite the long visual access to feeders and waterers under near-constant light, body weights at ages ranging from 31 to 49 were lighter for 23L:1D broilers than those for birds exposed to at least 4 h of darkness (Schwean-Lardner et al., 2011). Moreover, the older the birds were marketed, the more darkness could be added to the day and still result in higher body weights than broilers given near-constant light. Birds on near-continuous light were also less feed efficient, and had higher levels of mortality and leg disease than birds given more extensive darkness exposure.

Light is perceived in birds differently than in mammalian species, as it can either penetrate to the brain via the retina or, under higher light intensities, through the skull and directly to the hypothalamus (Prescott et al., 2003). It plays an important part in many regulatory activities within the body of a bird (Kristensen et al., 2006), and these may impact behaviour. However, when lighting programs are used, the effect on the behaviour of the bird is not usually one of the decision factors used to select a program (Mauldin and Graves, 1984).

There has been concern about the effect of long daylength lighting programs on the sleep patterns of broilers, and whether this is a welfare challenge for birds (Blokhuys, 1983). The potential for decreased welfare could be the result of either a reduced quantity or quality (Chen and Kushida, 2005) of sleep due to minimal dark periods and the potential for constant interruption of sleep by other birds. The latter could disrupt diurnal patterns, which is thought to

occur when continuous light is used (Manser, 1996). Sleep itself is partially regulated through a circadian rhythm pattern (Jones et al., 2008) and a disruption of diurnal patterns would result in a lack of rhythm synchrony and therefore sleeping birds being woken by birds that are awake. Although the functions of sleep are still poorly understood, it is thought that sleep is important in the restorative process of the brain and body physiological systems (Boerema et al., 2003), and deprivation of sleep has been shown to have numerous physiological consequences (Rechtschaffen et al., 1983). These can include drowsiness, leading to reduced alertness (Golub, 2000; Boerema et al., 2003), a reduction in ability to learn (Chun-Lin et al., 2004), a negative energy metabolism, in which food intake increases but body weight declines (Dinges, 2001; Rechtschaffen et al., 1983), and muscular weakness leading to death (Rechtschaffen et al., 1983).

Behaviour may be one of the most important indicators of animal welfare (Duncan, 1998), and evaluating behaviour can give us important clues to the animal's state. It is a technique that is non-invasive and, with the use of video camera systems, allows behavioural monitoring without human disruption to the animal (Dawkins, 2004). So what should be looked for? There are a number of types of behaviour that can help us determine the state of the animal. For example, stereotypes, which are frequently and repetitively performed behaviours that do not tend to fit the situation (Broom, 1988), indicate abnormality. The nature or lack of responsiveness to particular stimuli can indicate the animal's inability to interact in its environment (Broom, 1988). Changes to nutritive patterns might indicate a change in welfare, although as pointed out by Dawkins (1990) they may also indicate a different stage of an animal's life, such as hens during incubation periods. However, caution must be taken in the interpretation of behaviours. For example, in some negative situations there may actually be an increase in the performance of the comfort behaviour. An example by Mills (2010) was found in the increase in scratching and preening in an environment high in parasites. Duncan and

Mench (1993) have suggested that the presence of behaviours such as play and exploration (termed luxury behaviours) may aid in the positive assessment of welfare. Locomotory behaviours can indicate leg health, and a lack of motion can be due either to lack of motivation to move or an increase in leg disorders (Cooper, 2008). The amount of exercise required to impact leg health is not clear and research has frequently failed to establish a relationship between increased exercise and a lower incidence of leg disorders. Finally, fear can reduce the welfare state of birds, as it is considered to be a signal of emotional suffering (Jones, 1996). Work of Jones and Faure (1981) has shown that the latency to rise from a tonic state gives a strong indication of that fear level (tonic immobility).

Behaviour has been included as an assessment tool in the evaluation of darkness exposure in previous broiler lighting research. For example, Sanotra et al. (2002) compared birds with 8 h exposure to dark for most of their lives to those exposed to continuous light, and found that activity was higher for birds given darkness exposure. Behaviour in this work was measured only during a portion of the photoperiod. May and Lott (1992) found that birds raised under continuous light were not able to anticipate feeding periods as compared to birds given a dark period, strengthening the thought that light is an important zeitgeber in the establishment of circadian rhythms. More recently, Ferrante et al. (2006) used video camera analysis to observe behaviour of broilers under two lighting programs (24 h and 16 h) and two bird densities, finding a lack of feeding rhythm in birds reared on constant light.

The objective of this work was different than previous research. Its focus was to understand how graded increments of daylength and darkness exposure impact broiler behaviour and welfare during the overall 24 h day, including both light and dark periods.

MATERIALS AND METHODS

Approval of all experimental protocols used in this work was granted 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.

Experiments were conducted to examine the effect of daylength, bird genotype and bird gender on productivity traits in modern commercial broilers. These experiments have been previously described in detail (Schwean-Lardner et al., 2011). Four lighting programs were tested, using graded levels of darkness to allow regression relationships to be examined. The lighting programs were 14L:10D (14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L). All lighting programs were initiated on d 7; prior to this time, birds were maintained at 23L:1D. Light intensity was similar in all rooms (20 lux to d 7, then 10 lux for the remainder of the trial), with light provided by incandescent bulbs. Within each trial, eight rooms of broilers were used, allowing two room replications per lighting program. Nested inside each room were 12 straw litter floor pens, allowing sexes and genotypes (Ross x Ross 308, Ross x Ross 708) to be housed separately. Broiler housing and management approximated commercial conditions.

Detailed behavioural analyses were conducted within two trials of this work. With the use of an infrared lighting system and video recording systems, bird behaviour in one pen of Ross x Ross 308 male broilers (N=53 per pen) was captured with the use of a ceiling mounted video camera system and then digitalized to a computer system for a full 24 h for each lighting program. The camera was able to capture the entire area of the pen. Behaviour was assessed in one room per treatment on consecutive d in both experiments, thereby resulting in observation of both daylength replicates in each trial. In trial one, behaviour was recorded at 27 and 28 d (27 d) of age, and in trial two at 42 and 43 d (42 d) of age.

Data collection

The video data were observed (Genetec Omnicast Live Viewer 3.5) using a scan-sampling technique. Observations were made at 10-min intervals for the 24-h period (24 h x 6 measurements per h). For each time measured, the video frame was stopped and the behaviour of each bird recorded in one of the predetermined categories. Behaviours assessed were inactive resting (lying on the straw not performing any other behaviours – birds may or may not have been sleeping), walking, standing (standing and not performing any other behaviour), running, preening (manipulating feathers on own body, either lying or standing), leg or wing stretching, dustbathing, pecking or foraging at the litter, at the feeder (at the feeder with head into the lip of the feeder), and at the drinker (standing with head directly under the drinker line). The data were expressed as percent of time performing the various behaviours.

Response to observer was tested at 30 d of age in trial 1 and 47 d of age in trial 2. This was performed by one observer quietly walking into a room and past the pens of birds, counting the number of birds that made a physical motion away from the observer. This number was then converted to percentage of birds in the pen. Data were collected for each pen in the room, and averaged to create one value per room; two replicate rooms were tested per lighting program within each trial.

The relationship between daylength and fear was tested using tonic immobility at 33 d of age in trial 2. Eight birds per gender x genotype x lighting program were randomly selected and tested in a quiet hallway outside of bird rooms using the technique of Jones and Faure (1981). The tonic state was induced by holding birds in an inverted position for 15 s on a wooden, U shaped saddle. The latency to rise, measured in seconds (s) was recorded. Birds were re-tested if latency to rise was less than 10 s, and the maximum latency to rise allowed was 600 s.

Level of noise was measured once at 35 d of age, in two room replications for each treatment in trial 1. Level of noise was observed at approximately 6 h after the lights came on to

ensure noise was not associated with waking and feeding behaviour. An observer entered the room, and walked to the end of the room and back to the entrance door. A decibel reader (Radio Shack Sound Level Meter, Model 33-2050; Intertan INC., Barrie, Canada L4M 4W5) was used to measure the level of sound 6 times for each room, and a room average was then taken. As birds can react to individuals differently, the same observer measured all rooms in a random order.

Statistical analyses

The main effect in this work was lighting program. Behavioural percentage data was tested for normality using the Proc Univariate test of SAS (SAS[®] 9.1., Cary, NC). Because the majority of data did not follow a normal distribution, transformation of all data took place using (log+1) transformation (Steele and Torrie, 1980) prior to analysis. The data were then tested for regression relationships with daylength using Proc Reg and Proc RSReg of SAS.

RESULTS

Daylength impacted behaviour regardless of age in a similar fashion. In general, increasing daylength resulted in a decline in activity and other behaviours.

Inactivity – resting

The level of listlessness or inactivity is sometimes used as one of a group of behaviours that help define animal welfare. In particular, increases in this parameter may indicate poor welfare, potentially due to pain or other causes. In this work, increasing daylength resulted in birds spending an increasing percentage of time, in a linear fashion, in inactive resting regardless of the age measured (27 d or 42 d) (Table 1) during the photoperiod. Broilers were mostly inactive during the dark period, but the relationship between this behaviour and daylength is still statistically significant during the scotoperiod primarily due to more activity in

birds given 14L. When data are summarized over a full 24-h period, a positive linear relationship exists again at both 27 and 42 d, although it nears a quadratic response ($P=0.07$) at 42 d.

While the regression analyses for the photoperiod and 24-h periods show a linear response, 17L birds spend less or the same amount of time in inactive resting than do broilers given 14 h of light.

Mobility behaviours

Table 2 shows the relationships between daylength and mobility behaviours, including standing, walking and running. Daylength impacts the percentage of time birds spend standing, and the results are similar in both age groups. During the light period, the effect is linear, and birds receiving the longest daylength stand the least. In both age groups, birds on 14 and 17-h light stand a similar amount of time and as a result a quadratic relationship approaches significance ($P=0.09$). Standing during the scotoperiod occurred at a low level; however, the linear effect was maintained. In this case, birds on near-constant photoperiods do not stand; once darkness is given to these birds, they perform no other activities. On the other end of the daylength scale, birds at 42 d on long dark periods (14L) show the most standing activity during the dark period. When averaged over a full 24-h period, standing decreased linearly with increasing daylength at 27 d. At 42 d, the effect is similar and nears quadratic significance (0.09), again with birds exposed to longest daylengths standing the least throughout the day.

Walking, an exercise behaviour, was also impacted by daylength (Table 2). There was a significant linear regression between daylength and the percentage of time spent walking, regardless of age and whether measured during the photoperiod, scotoperiod, or averaged over 24 h. In essence, as days get longer, less walking takes place. Birds receiving 23L per day walked the least, which coincides with the increased level of inactive resting as discussed above. Although statistics indicate a linear effect, levels of walking were either significantly

higher (27 d) or similar (42 d) for broilers in the 17L treatment as compared to those given 14L, resulting in a quadratic relationship to daylength.

The effect of daylength on running behaviour is less distinct. Regression analysis failed to demonstrate a significant effect ($P \leq 0.05$), but a comparison of means using Duncan's Multiple Range Test showed that 17L broilers ran the most during the photoperiod and for the entire 24 h at 27 d. Running did not occur during any period or at any age under near-constant light.

Nutritive behaviours

Feeding and drinking behaviours are functionally necessary behaviours, required for livability. Selection for increased growth in commercial broiler stock has likely caused enhanced motivation to feed, resulting in higher feed intakes and heavier body weights at younger ages. Therefore, it is interesting to establish how daylength can impact this motivation and nutritive intake.

Table 3 demonstrates the effect of daylength on feeding and drinking behaviours. During the photoperiod at 27 d, feeding demonstrated a quadratic relationship with daylength. Birds on long days spent less time at the feeder than those on shorter days. On the other end of the daylength spectrum, a peak in percentage of time spent at the feeder occurred under 17L. Birds on slightly shorter days (14L) did not spend a higher percentage of their time at the feeders even though their days are shorter. Scotoperiod feeding behaviour also showed a linear relationship with daylength, although this is misleading. The only birds that were at the feeder during the night period were those on 14L. When data are averaged over 24 h, a quadratic relationship for feeding behaviour still exists with daylength with the peak feeding time occurring under 17L. Daylength did not change the percentage of time that bird spent at the drinker.

The data for feeding behaviour at 42 d are similar to those from younger birds (Table 3). During the photoperiod, feeding behaviour decreased in a linear fashion with increasing

daylength. The relationship between time at the feeder and daylength during the dark period is quadratic, but once again this is misleading. The only birds at the feeder during the scotoperiod were those on the 14L treatment. Averaged overall, birds raised on 17L spent the highest percentage of time at the feeder and those given 23L the least, resulting in a quadratic relationship.

Comfort, maintenance and exploratory behaviours

Preening

Preening is a body maintenance behaviour, important in keeping feather health at its best. It has also been grouped within the comfort behaviour category. At 27 d (Table 4), preening decreased linearly with increasing daylength during the photoperiod and averaged over a 24-h period. No preening was noted during the scotophase. At 42 d, a similar but quadratic relationship was seen during the photoperiod and averaged over 24 h. Again, lowest levels were noted when birds are raised under near-constant photoperiods, and levels were lower under 14L as compared to 17L. Only birds from the 14L treatment preened during the dark period and this resulted in a linear statistical interpretation of the effect of daylength.

Leg or wing stretching

Stretching of the wings or legs is considered a comfort behaviour. Once again, photoperiod affects the percentage of time that is spent stretching (Table 4). Regardless of age, the effect seen under the photoperiod and the 24 h average is linear, although quadratic regressions are nearing significance at 42-43 d of age during the photoperiod ($P=0.07$) and 24 h average ($P=0.07$). Birds raised under near-constant photoperiods do not stretch as much as birds under any other photoperiod. Stretching was seen during the scotoperiod of birds from the 14L treatment at 42 d and this resulted in a linear relationship between daylength and this behaviour.

Dustbathing

Dustbathing, an exercise (Shields et al., 2004) and a feather maintenance behaviour, decreased in a linear fashion with increasing daylength at 27 and 42 d (Table 4) and was not observed in 23L birds at 42 d. Dustbathing was not observed during the scotophase for any treatments.

Litter foraging

Litter foraging was affected by daylength in a quadratic or near-quadratic fashion (Table 4) regardless of age measured, during the photoperiod and over the 24-h period with the least foraging occurring under 23L. At 42 d, a low level of foraging was noted for birds from the 23L treatment.

Response to observer

The response to observer data (Table 5) was performed at 30 and 47 d of age. A quadratic relationship with the percent of birds responding to an observer and daylength was found. Birds exposed to 23L were the least reactive regardless of age.

Tonic immobility

Tonic immobility was measured at 33 d of age. No significant differences were found in the latency to rise (Table 6).

Sound

Birds raised on 23L were quieter than those raised on any other photoperiod program (Table 7).

DISCUSSION

Although the environment in which broilers are raised commercially is very different from that of their wild ancestors, poultry have maintained a similar behavioural repertoire (Jurkevish and Grossmann, 2002). Therefore, it can be expected that although levels of behaviours may be different, the behaviours themselves still exist. It is important to examine what behaviours are performed and to what extent, because behavioural activities give us an indication of what is happening in an animal's life. Without an understanding of the function, importance or strength of the motivation for a behaviour, it may be difficult to assess welfare on this aspect alone. Welfare is not necessarily compromised if the incidence of a particular behaviour is reduced in frequency, but welfare may suffer if the frequency of highly motivated behaviour is reduced or eliminated (Dawkins, 1990; Duncan, 1998; Prescott et al., 2003). This may also be true of behaviours that are functional necessities or have health implications, such as exercise or eating.

Exercise is important in the development of bone health (Robbins et al., 1984). In humans, both walking (Guadalupe-Grau et al., 2009) and standing (Chad et al., 1999) have been shown to result in enough load-bearing work to be a contributing factor. Therefore, standing, walking and running behaviours may contribute to exercise and bone health. Because the incidence of leg weakness in broiler chickens is an often cited welfare issue, management factors that result in less time exercising may have a negative impact. This has occurred with the near-constant photoperiod. The introduction of 4 h of darkness improves this somewhat, but behaviour under that photoperiod tends to be intermediate between near-constant light programs and those with longer dark periods.

The lack of movement seen by birds on long photoperiods may also reduce welfare for reasons other than lack of exercise. It indicates a lack of motivation, and this will be shown as a reduction in behaviours that broilers are known to be motivated to perform. Lack of activity can

also increase levels of breast blisters, hock burns or foot pad lesions (Deaton, 1978; Gordon, 1994; SCAHAW, 2000), all of which can be painful and reduce the well-being of the birds. The elimination of running under near-constant light also indicates a welfare issue, as this behaviour can be termed play behaviour and can be involved in the exercise regime of a bird (Fraser and Broom, 1990). The inactivity or listlessness may, on its own, indicate a reduction in welfare. Fraser and Broom (1990) referred to extreme lethargy as an abnormal behaviour, and specify that one possible cause may be neural malfunctioning. It is interesting that this lack of movement may be a reason for producers preferring this photoperiod, as birds are easier to catch prior to marketing.

The list of behaviours that broilers, selected for maximal feed intake and growth rate, are motivated to perform is not extensive. Their desire to eat is undoubtedly strong (Bokkers et al., 2004), impacted through genetic selection for increased growth rate. Near-constant light unexpectedly reduced the amount of time spent feeding in this work, even when considered over a full 24-h period. Because there is no visual restriction caused by extended darkness under this photoperiod regime, there must be another reason why feeding activity is reduced. These include a reduced motivation to eat, heavier body weights, particularly early weights (Reiter and Bessei, 2001), or leg disease. In this work, market weight of birds raised under near-constant photoperiods were never the heaviest and at 48 d in fact weighed less than birds given 17 or 20 h of light and the same as birds exposed to 10 h of darkness (Schwean-Lardner et al., 2011). Finally, it is possible that unhealthy birds eat less, and mortality and morbidity in these studies were higher in birds raised under near-constant light. Regardless of the reason, the reduction in time spent at the feeder indicates a problem in birds raised on near-constant light. The relationship between time spent at the feeder and actual feed consumption (Schwean-Lardner et al., 2006a) is also of interest. Birds raised under 23L ate more than birds under 14L or 17L, yet birds with darkness exposure spent more time at the feeders. Based on

time spent walking, this might suggest that birds under 23L ate less frequently and larger meals, possibly because of a reluctance to move. The difference in the time spent at the feeder could indicate play or foraging activity around the feeder area in birds with darkness exposure. Very short daylengths, such as used in this work (14L), resulted in lower percentages of times at the feeder than did 17L on a 24 h basis, but this is understandable and explainable. Because the days were shorter, birds likely spent time performing all activities and may have been restricted in their feeding time. Interestingly, the group of birds under 14L were the only birds to show night time eating, suggesting that they were hungry before lights come on, and that they had reached a saturation point in sleep.

Welfare may be dependent on an animal's behavioural needs being met (Duncan, 1998). Luxury behaviours are those that occur when basic needs in an animal's life are met, and can include exploratory and play behaviours (Duncan and Mench, 1993; Duncan, 1998). Running, for example, showed the highest incidence at 28 d under 17L (photophase and total of 24 h) and, interestingly, this trend appears in a number of behaviours. It is very likely that shortening the day to 14L reduced the incidence of running or other behaviours because of the limited daylength and the need to perform necessary behaviours such as feeding and drinking. The 20L birds performed an intermediate level of running, again indicative that 4 h of darkness may not provide adequate resting time. Finally, the complete elimination of running under near-constant light, even in younger ages of birds, may indicate a welfare issue.

Animals are motivated to explore and forage (Wood-Gush and Vestergaard, 1991; Newberry, 1999; Shields et al., 2004), and a reduction or elimination of these behaviours, as well as body maintenance behaviours, is indicative of reduced welfare. A particular example is preening, which is necessary for feather maintenance and thought to occur sporadically throughout the day when other more basic needs have been met (Delius, 1988). In this work, linear or quadratic relationships existed where the most preening occurred under 14L or 17L,

the lowest level under near-constant light, and 20L resulting in an intermediate level of the behaviour. Again, this could be indicative of a reduction in welfare under 23L, and a suggestion that 20L is only providing enough darkness for an intermediate effect. Once again, similarities exist between the behavioural level seen under 14L and 17L programs for the reasons discussed above.

In terms of exploratory behaviours, Fraser (1988) pointed out that animals that have a mundane environment will show a reduced level of exploratory behaviour, but the behaviour will not be eliminated and the potential for strong motivation for such behaviour is still high (Fraser and Broom, 1990). In this work, however, birds raised on near-constant light perform little foraging behaviour, again hinting at a reduction in welfare, and birds raised under 20L again were intermediate in their behavioural expression.

Differences were also noted in time spent dustbathing. Dustbathing functions in a feather maintenance role and may play a role in increasing exercise as well (Shields et al., 2004). Again, the behaviour was nearly or completely eliminated under 23L at either age, performed at similar levels under 14L or 17L, and intermediate (although close to zero) under 20L.

Leg and wing stretching are also important behaviours, as stretching can help in maintaining functionality of joints and muscles (Fraser and Broom, 1990), and the reduction seen with increasing daylength may reduce health in birds raised on this photoperiod.

Birds on lighting programs are often thought to be more difficult to catch at market age, and producers attribute this to fear. There were no significant differences in tonic immobility in this work, suggesting fear levels themselves are not dependent on photoperiod, although numerically the latency to rise was longer in birds given a dark period. In rats, an association between sleep deprivation and poor fear memory has been noted (Chun-Lin et al., 2004), hence fearful situations were forgotten faster. Sleep deprivation could explain the numerical

differences noted. Results for lighting impact on tonic immobility in the literature do vary. Møller et al. (1998) and Campo et al. (2007) found birds raised on a constant photoperiod had longer latency to rise periods than birds given 14L, suggesting higher fear levels.

Vocalization in animals has been used as a welfare indicator (Manteuffel et al., 2004), and Fraser (1988) pointed out that sound in a livestock facility is indicative of motivation and reactivity in the animals. Vocalization is a communication tool amongst animals, and can be affected by a number of parameters, including environment and the awareness of the animal itself (Manteuffel et al., 2004). In this experiment, birds raised under photoperiods that included more than 1 h of darkness all produced similar sound levels, yet those raised under near-constant light were significantly quieter. This could be attributed to a lack of awareness from sleep deprivation and its effect on poor neural functioning. The data correspond well with the lack of activity in these birds.

In this research, long daylengths reduce welfare for broilers as expressed by behaviour, but it has not been established why this occurs. It has been suggested that health declines under this management program, which could result in the reduction in active behaviours. Health was impacted, and long photoperiods resulted in higher mortality and cull levels due to skeletal defects and poorer gait scores in the live flocks (Schwean-Lardner et al., 2006b). While this undoubtedly affects behaviour, there may be other factors that affect the changes in behavioural expression that were seen, although research in this area has been limited. It is possible that lack of or only short periods of darkness alter either the quantity or quality of sleep. This could be because the dark period is not present, although it has been shown that birds raised under constant light still sleep (Savory and Duncan, 1982; Ayala-Guerrero et al., 2003). The rhythm of light and dark within a day is thought to be one of the main regulatory factors involved in establishing poultry sleep patterns (Fraser and Broom, 1990). This zeitgeber acts as a signal for many behaviours that generally operate in a diurnal pattern, including sleep, and

allows the flock as a whole to follow a similar pattern. Therefore, disruption of this pattern could result in sleep deprivation because of the failure to develop circadian rhythms. Contrary to this hypothesis is the finding that birds can develop a sleep rhythm under constant light conditions (Ayala-Guerrero et al., 2003), possibly driven by other environmental zeitgebers, such as temperature and/or external disturbances (Blokhus, 1984). However, if this occurs, it would likely exist on an individual bird basis and, because the diurnal pattern within the flock would no longer be synchronized, could still lead to disruption of birds sleeping while other birds, following a different circadian pattern, are performing other behaviours. This would lead to a poorer quality of sleep. In this work, a similar disruption of the release of the hormone melatonin, which has a strong relationship with sleep, was found in birds raised under 23L (Schwean-Lardner et al., 2010). In humans, regardless of how sleep deprivation occurs the result on daytime productivity is similar, and symptoms include a reduction in reactivity to the extent where reaction times are actually poorer than in those who are legally alcohol impaired (Powell et al., 2001). Sleep deprivation can also impair neural activity (Fraser and Broom, 1990), resulting in a disruption of synaptic activity within the brain (Chen and Kushida, 2005). The result of this combination of reduced brain function and poor reactivity could result in lethargic birds that perform less exercise, nutritive, comfort, exploratory and other behaviours. Sleep deprivation has also resulted in rats that have poor fear memory (Chun-Lin et al., 2004) and a reduced arousal reflex (Thakker et al., 2003), which would result in an alteration to their response to observer as shown in the data presented in this study.

While it may be difficult to base a welfare assumption on one behaviour alone, examining a large number of behaviours over the entire day can give a strong indication on its state. Behaviour in this research was impacted by daylength, and it is interesting to note that, generally, the effect was similar. Birds on 23L are generally inactive and perform most behaviours the least amount of time. This is an unexpected result considering the birds have

unlimited visual access to their environment. The elimination or near elimination of behaviours is also a concern, as is poor reactivity to humans and the reduction in communication that takes place in the flock. Therefore, not allowing birds to have a dark period in which sleep can be undisturbed reduces broiler welfare. Birds raised under 20L tend to be intermediate in their behavioural expression, and could suggest that 4 h of darkness is not enough to maximize behavioural activity. Also of interest is that birds under 17L perform many behaviours and communication levels for a larger percentage of time as compared to birds under 14L, but a simple restriction of time under the 14L photoperiod may explain this trend. There are also a number of suggestions from this data that point to birds on 10 h dark periods likely having more darkness than required, as indicated by the birds waking and performing behaviours other than resting during the dark period. This included night time eating, as mentioned above, standing at night time, and an increase in preening activity during the dark period.

Finally, the question of what photoperiod length maximizes welfare as measured by behaviour is of interest. Examining the behavioural data by combining both ages and calculating the regression equation gives a strong indication, as shown in Table 8. The data for the behaviours shown satisfy a quadratic relationship ($P < 0.10$), with the exception of eating. Calculating the predicted values for these behaviours indicates that the active behaviours are maximized at 16 or 17 h of light per day. Because the significantly quadratic behaviours listed are comfort, body maintenance, and exercise behaviours, it is likely that at this daylength welfare is at its best, as judged by behaviour.

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TABLE 4.1. The effect of daylength on broiler inactive resting as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
<u>27/28 d</u>								
Photoperiod								
Log+1	4.08 ^B	4.00 ^B	4.30 ^A	4.36 ^A	0.060	0.0170	Y=3.4905+0.0378x	0.64
Original	58.78	53.81	73.01	77.45	3.885	-	-	-
Scotoperiod								
Log+1	4.59	4.61	4.61	4.62	0.004	0.0187	Y=4.5548+0.0028x	0.63
Original	97.48	99.13	99.87	100	0.441	-	-	-
24 h mean								
Log+1	4.28 ^B	4.22 ^C	4.36 ^A	4.37 ^A	0.024	0.0287	Y=4.0399+0.0145x	0.58
Original	71.01	67.03	77.48	78.39	1.789	-	-	-
<u>42/43 d</u>								
Photoperiod								
Log+1	4.07 ^B	4.06 ^B	4.34 ^A	4.47 ^A	0.067	0.0013	Y=3.3292+0.0489x	0.84
Original	57.53	57.18	75.45	85.99	4.726	-	-	-
Scotoperiod								
Log+1	4.59 ^B	4.61 ^A	4.61 ^A	4.62 ^A	0.004	0.0302	Y=4.5667+0.0023x	0.57
Original	97.67	99.94	99.67	100	0.376	-	-	-
24 h mean								
Log+1	4.32 ^{BC}	4.26 ^C	4.39 ^{AB}	4.47 ^A	0.031	0.0210	Y=3.9990+0.0195x	0.62
Original	74.25	69.73	79.49	86.58	2.470	-	-	-

¹SEM – Standard error of the mean

²Values for P (probability of regression), regression equation and R² based on log-transformed values.

TABLE 4.2. The effect of daylength on broiler mobility as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (hours of light per day)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
<u>27/28 d</u>								
Standing								
Photoperiod								
Log+1	2.02	2.24	1.72	1.40	0.126	0.0163	Y=3.3219-0.0799x	0.65
Original	6.60	8.38	4.59	3.09	0.785	-	-	-
Scotoperiod								
Log+1	0.45	0.51	0.13	0	0.085	0.0057	Y=1.3466-0.0581x	0.75
Original	0.59	0.67	0.13	0	0.115	-	-	-
24 h mean								
Log+1	1.74	1.96	1.58	1.36	0.089	0.0430	Y=2.5996-0.0507x	0.52
Original	4.71	6.13	3.84	2.96	0.460	-	-	-
Walking								
Photoperiod								
Log+1	1.61	1.85	1.21	0.94	0.135	0.0109	Y=3.0312-0.0881x	0.69
Original	4.01	5.35	2.36	1.56	0.567	-	-	-
Scotoperiod								
Log+1	0.17	0.14	0	0	0.035	0.0020	Y=0.4788-0.0217x	0.62
Original	0.19	0.16	0	0	0.039	-	-	-
24 h mean								
Log+1	1.34	1.57	1.09	0.91	0.097	0.0243	Y=2.3231-0.0591x	0.60
Original	2.83	3.84	1.97	1.49	0.346	-	-	-

Running

Photoperiod

Log+1	0.29	0.54	0.06	0	0.082	0.0553	$Y=1.0575-0.0451x$	0.48
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Original	0.34	0.71	0.07	0	0.111	-	-	-
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Scotoperiod

Log+1	0	0	0	0	0	NS	-	-
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Original	0	0	0	0	0	-	-	-
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24 h mean

Log+1	0.21	0.41	0.05	0	0.061	NS	-	-
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Original	0.23	0.51	0.06	0	0.077	-	-	-
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42/43 d**Standing**

Photoperiod

Log+1	1.60	1.57	1.58	0.88	0.125	0.0384	$Y=2.7460-0.0724x$	0.54
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Original	4.02	3.81	3.85	1.45	0.435	-	-	-
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Scotoperiod

Log+1	0.81	0.06	0.28	0	0.128	0.0407	$Y=1.6459-0.0734x$	0.53
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Original	1.29	0.06	0.33	0	0.215	-	-	-
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24 h mean

Log+1	1.35	1.31	1.45	0.85	0.096	0.0928	$Y=-2.9853+0.5212x-0.0153x^2$	0.66
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Original	2.88	2.71	3.26	1.39	0.287	-	-	-
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Walking								
Photoperiod								
Log+1	1.51	1.55	1.05	0.42	0.175	0.0076	$Y=-2.7857+0.5723x-0.0189x^2$	0.96
Original	3.54	3.74	1.87	0.53	0.507	-	-	-
Scotoperiod								
Log+1	0.08	0	0	0	0.013	0.0041	$Y=0.9040-0.0903x+0.0022x^2$	0.93
Original	0.08	0	0	0	0.014	-	-	-
24 h mean								
Log+1	1.13	1.29	0.94	0.40	0.129	0.0042	$Y=-3.9012+0.6310x-0.0193x^2$	0.95
Original	2.10	2.64	1.56	0.50	0.307	-	-	-
Running								
Photoperiod								
Log+1	0.03	0.16	0.06	0	0.029	NS	-	-
Original	0.03	0.18	0.07	0	0.032	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	0.02	0.11	0.05	0	0.022	0.0926	$Y=-1.2736+0.1517x-0.0042x^2$	0.49
Original	0.02	0.12	0.05	0	0.023			

¹ SEM – Standard error of the mean

² Values for *P* (probability of regression), regression equation and *R*² based on log-transformed values.

TABLE 4.3. The effect of daylength on broiler nutritive behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
<u>27/28 d</u>								
At feeder								
Photoperiod								
Log+1	2.51	2.63	2.40	2.15	0.069	0.0163	Y=-0.2357+0.3429x-0.0104x ²	0.89
Original	11.30	12.87	10.08	7.61	1.010	-	-	-
Scotoperiod								
Log+1	0.96	0	0	0	0.169	0.0436	Y=10.8098-1.0794x+-0.0266x ²	0.80
Original	1.75	0	0	0	0.333	-	-	-
24 h mean								
Log+1	2.22	2.31	2.24	2.11	0.031	0.0400	Y=0.4961+0.2073x-0.0060x ²	0.72
Original	8.27	9.12	8.40	7.29	0.287	-	-	-
At drinker								
Photoperiod								
Log+1	2.14	2.26	1.90	1.91	0.073	NS	-	-
Original	7.65	8.64	5.68	5.75	0.990	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	1.83	1.96	1.74	1.87	0.040	NS	-	-
Original	5.23	6.12	4.74	5.51	0.267	-	-	-

TABLE 4.3. The effect of daylength on broiler nutritive behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
42/43 d								
At feeder								
Photoperiod								
Log+1	2.55	2.45	2.09	1.82	0.111	0.0001	Y=3.8073-0.0853x	0.96
Original	11.82	10.64	7.11	5.16	1.010	-	-	-
Scotoperiod								
Log+1	0.43	0	0	0	0.072	0.0124	Y=4.8517-0.4845x+0.0119x ²	0.89
Original	0.54	0	0	0	0.092	-	-	-
24 h mean								
Log+1	2.09	2.14	1.94	1.78	0.054	0.0259	Y=0.8443+0.1686x-0.0056x ²	0.94
Original	7.12	7.51	5.93	4.94	0.384	-	-	-
At drinker								
Photoperiod								
Log+1	2.48	2.39	1.98	1.80	0.115	0.0020	Y=3.6802-0.0820x	0.82
Original	11.01	9.94	6.38	5.06	0.990	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	2.00	2.08	1.83	1.76	0.063	0.0836	Y=2.5155-0.0323x	0.42
Original	6.42	7.02	5.32	4.85	0.422	-	-	-

¹SEM – Standard error of the mean

²Values for P (probability of regression), regression equation and R² based on log-transformed values.

TABLE 4.4. The effect of daylength on broiler exploratory, luxury and maintenance behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
<u>27/28 d</u>								
Preening								
Photoperiod								
Log+1	1.49	1.42	1.03	0.75	0.114	0.0001	Y=2.7816-0.0869x	0.92
Original	3.45	3.15	1.81	1.12	0.365	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	1.23	1.17	0.92	0.73	0.078	0.0005	Y=2.0832-0.0579x	0.88
Original	2.42	2.23	1.51	1.08	0.215	-	-	-
Leg or wing stretching								
Photoperiod								
Log+1	0.45	0.93	0.13	0.01	0.135	0.0767	Y=1.6774-0.0702x	0.43
Original	0.56	1.54	0.13	0.01	0.229	-	-	-
Scotoperiod								
Log+1	0	0.04	0	0	0.010	NS	-	-
Original	0	0.04	0	0	0.010	-	-	-
24 h mean								
Log+1	0.33	0.74	0.11	0.01	0.107	0.0954	Y=1.2754-0.0529x	0.39
Original	0.39	1.10	0.11	0.02	0.162	-	-	-

TABLE 4.4. The effect of daylength on broiler exploratory, luxury and maintenance behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
Dustbathing								
Photoperiod								
Log+1	0.47	0.59	0.17	0.15	0.079	0.0345	Y=1.2031-0.0464x	0.55
Original	0.60	0.82	0.19	0.16	0.119	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	0.34	0.45	0.14	0.14	0.056	0.0548	Y=0.8407-0.0308x	0.49
Original	0.41	0.58	0.16	0.15	0.077	-	-	-
Litter foraging								
Photoperiod								
Log+1	1.49	1.73	1.12	0.33	0.205	0.0057	Y=-5.7943+0.9250x-0.0287x ²	0.95
Original	3.48	4.72	2.08	0.39	0.637	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	1.23	1.46	1.00	0.32	0.165	0.0033	Y=-5.4992+0.8402x-0.0256x ²	0.95
Original	2.41	3.34	1.74	0.38	0.427	-	-	-

TABLE 4.4. The effect of daylength on broiler exploratory, luxury and maintenance behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
<u>42/43 d</u>								
Preening								
Photoperiod								
Log+1	1.67	1.95	1.34	0.75	0.172	0.0162	Y=-4.4953+0.7818x-0.0242x ²	0.91
Original	4.32	6.08	2.86	1.13	0.709	-	-	-
Scotoperiod								
Log+1	0.18	0	0	0	0.036	0.0817	Y=0.3861-0.0184x	0.42
Original	0.21	0	0	0	0.041	-	-	-
24 h mean								
Log+1	1.28	1.66	1.21	0.73	0.129	0.0123	Y=-5.3919+0.8140x-0.0239x ²	0.86
Original	2.60	4.30	2.38	1.08	0.451	-	-	-
Leg or wing stretching								
Photoperiod								
Log+1	0.45	0.49	0.29	0.12	0.057	0.0041	Y=1.0613-0.0392x	0.72
Original	0.57	0.63	0.33	0.13	0.079	-	-	-
Scotoperiod								
Log+1	0.15	0	0	0	0.028	0.0643	Y=0.3183+0.0151x	0.46
Original	0.17	0	0	0	0.031	-	-	-
24 h mean								
Log+1	0.34	0.37	0.24	0.12	0.039	0.0092	Y=0.7440-0.0258x	0.70
Original	0.40	0.45	0.28	0.13	0.051	-	-	-

TABLE 4.4. The effect of daylength on broiler exploratory, luxury and maintenance behaviours as a percentage of time over the photoperiod, scotoperiod and 24-h time periods

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
Dustbathing								
Photoperiod								
Log+1	0.35	0.39	0.02	0	0.079	0.0281	Y=1.0695-0.0475x	0.58
Original	0.45	0.48	0.02	0	0.103	-	-	-
Scotoperiod								
Log+1	0	0	0	0	0	NS	-	-
Original	0	0	0	0	0	-	-	-
24 h mean								
Log+1	0.22	0.29	0.02	0	0.055	0.0427	Y=0.7162-0.0315x	0.52
Original	0.26	0.34	0.02	0	0.066	-	-	-
Litter foraging								
Photoperiod								
Log+1	1.66	2.10	1.10	0.44	0.242	0.0140	Y=4.2039-0.1556x	0.66
Original	4.35	7.31	2.06	0.56	1.031	-	-	-
Scotoperiod								
Log+1	0.04	0	0	0	0.010	NS	-	-
Original	0.04	0	0	0	0.010	-	-	-
24 h mean								
Log+1	1.26	1.80	0.99	0.42	0.195	0.0450	Y=-6.9802+1.0233x-0.0306x ²	0.80
Original	2.55	5.17	1.72	0.53	0.693	-	-	-

¹ SEM – Standard error of the mean

² Values for P (probability of regression), regression equation and R² based on log-transformed values.

TABLE 4.5. The effect of daylength on percent of birds moving away from observer's presence

	Daylength (h)				Pooled SEM ¹	P value ²	Equation	R ²
	14	17	20	23				
30 d								
Log+1	3.05	3.32	3.33	1.37	0.153	0.0001	$Y=-14.7680+2.138x-0.062x^2$	0.71
Original	21.30	26.99	27.34	4.18	1.776	-	-	-
47 d								
Log+1	3.61	3.28	2.97	1.54	0.175	0.0179	$Y=-3.198+0.908x-0.030x^2$	0.64
Original	38.71	28.99	22.15	4.52	3.050	-	-	-

¹SEM – Standard error of the mean

²Values for *P* (probability of regression), regression equation and R² based on log transformed values.

TABLE 4.6. The effect of daylength on tonic immobility latency to rise (seconds)

	Daylength (h)				Pooled SEM ¹	<i>P</i> value ²	Equation	R ²
	14	17	20	23				
Latency to rise (sec)	217.72	230.9	234.1	172.9	16.95	NS	-	-

¹ SEM – Standard error of the mean

² Values for *P* (probability of regression), regression equation and R² based on log-transformed values.

TABLE 4.7. The effect of daylength on sound – 47 d (decibels)

	Daylength (h)				Pooled SEM ¹	<i>P</i> value ²	Equation	R ²
	14	17	20	23				
Sound	60.25	62.13	68.50	51.75	2.400	0.0391	$Y=-98.797+18.505x-0.517x^2$	0.65

¹SEM – Standard error of the mean

²Values for *P* (probability of regression), regression equation and R² based on log-transformed values.

TABLE 4.8. Predicted values for percent of time birds are performing behaviours over 24-h period – averaged over 2 ages. The peak in each behavioural expression is highlighted (exception is resting where the low value is highlighted).

Daylength (h)	Behaviours							
	Resting	Walking	Standing	Running	Preening	Stretching	At the feeder	Foraging
14	71.6	2.54	3.76	0.15	2.62	0.48	7.84	2.71
15	71.09	2.68	4.00	0.19	2.79	0.54	8.02	3.09
16	71.04	2.73	4.13	0.20	2.86	0.57	8.11	3.30
17	71.44	2.69	4.14	0.22	2.85	0.58	8.11	3.35
18	72.31	2.57	4.05	0.20	2.75	0.56	8.02	3.22
19	73.63	2.36	3.84	0.18	2.56	0.51	7.83	2.92
20	75.41	2.06	3.52	0.15	2.28	0.43	7.55	2.45
21	77.65	1.68	3.09	0.10	1.91	0.32	7.18	1.82
22	80.34	1.21	2.55	0.03	1.45	0.19	6.72	1.01
23	83.50	0.66	1.89	-0.04	0.90	0.03	6.17	0.03
<i>P</i> value of quadratic	0.0595	0.0201	0.0989	0.1247	0.0216	0.0591	0.1759	0.0003

Preface to Chapter 5: The effect of daylength on flock behavioural and melatonin rhythms in broilers

Many of the results from Chapters two through four have led us to hypothesize that birds on long photoperiods are sleep deprived. However, current literature has not explained how this occurs. It has been shown that birds can sleep during the light period, so likely total sleep deprivation is not the problem. This chapter hypothesized that broiler flocks on long daylengths do not form synchronized behavioural patterns throughout the day. If this is the case, then sleep fragmentation (repeated awakenings) may occur. Melatonin, a neurohormone involved in sleep promotion, is also examined with regards to flock synchronization.

Chapter 5. The effect of daylength on flock behavioural and melatonin rhythms in broilers^{*}

^{*} A version of this chapter has been submitted for publication to Animal Welfare. Schwean-Lardner, K., B.I. Fancher, B. Laarveld, and H.L. Classen. November 2011.

ABSTRACT Biological patterns or rhythms allow synchrony to develop between external and internal variables, including between time and behavioural activities. Ross x Ross 308 male broilers (30 kg/m²) were used to study the impact of daylength (14, 17, 20 and 23L) on photophase flock behavioural 24 h melatonin rhythms. Using infrared cameras, behaviour in two pens of 53 birds per lighting treatment was continuously recorded for a full 24 h in trial one (27-28 d of age (d 27)) and two (42-43 d (d 42)). Behavioural expression was quantified using a scan-sampling technique every 10 min for the full 24 h daylength. Proc Reg and RSReg of SAS were used to test trend analysis in the flock between behavioural variables and time for the photoperiod phase. To determine the presence of a biological rhythm in flock melatonin production, blood samples were collected six times for birds raised on 23L and eight times for 14, 17 and 20L birds (n=6 per time) over a 24-h period (d 21), and analyzed using radioimmunoassay analysis. Quadratic (linear where noted) relationships between time and behaviour during the photophase ($P \leq 0.05$) were frequent when birds were raised on 14L and 17L, sporadic under 20L, and non-existent under 23L. This included inactive resting (27 d: 14, 17L; 42 d: 14, 20L), where the fewest birds were resting at the start and just prior to the end of the light period; walking (27 d: 14, 17L), standing (27 and 42 d: 14, 17, 20L), feeding (27 d: 14, 17L), and drinking (27 d: 14, 17, 20L), where peaks existed at start and end of photophase; and preening (27 d: 14, 17L) and dustbathing (27 d: 14, 17, 20L), with the highest frequency occurring mid-photophase. A quadratic time x melatonin relationship existed in 14, 17 and 20L flocks, with the highest production during the scotoperiod suggesting synchrony of this circadian rhythm within the flock. No relationship existed in 23L flocks. In conclusion, behaviour during the photophase and melatonin production over a 24-h period indicates that 23L flocks do not develop synchronized rhythms. This increases the possibility that birds raised under this photoperiod are interrupted when attempting to sleep, leading to sleep fragmentation.

Key words: light, dark, melatonin, circadian rhythm, sleep, welfare

INTRODUCTION

Circadian rhythms, or chemical, biological or behavioural functions that appear with an approximate frequency of 24 h, play an important part in the well-being of an animal. Development of these rhythms ensures that functions are synchronized and co-ordinated with each other (Ohta et al., 2005). Although organisms have endogenous clocks that create rhythm based on either daily, tidal, lunar or even annual cycles (Menaker, 2002), there are often external cues that help to drive a rhythm, and keep it within a specific time range. Light (specifically dawn and dusk) is one of the primary “zeitgebers” that helps to maintain biological, physiological or behavioural rhythms within a norm of approximately 24 h for many animals including birds (Fleissner and Fleissner, 2002).

In birds, melatonin is produced in a rhythmic fashion in both the pineal gland and the retina, and its importance cannot be downplayed. Melatonin is one of the primary factors maintaining and regulating circadian rhythms within the body (Brandstätter, 2002). In terms of a daily rhythm, melatonin is produced during the dark period (Pang et al., 1996), and light itself helps to repress the production of melatonin during the day (Appleby et al., 2004). Hence, the normal production of melatonin provides peaks of the hormone during the scotoperiod and valleys during the photoperiod.

Many behavioural functions also occur on a 24 h rhythm. For example, rest and/or sleep occur primarily during the dark period (Appleby et al., 2004). Other behaviours are slightly more complicated. For example, feeding occurs primarily during the day, but even within the photoperiod section of the day a rhythm or pattern typically develops. Birds eat when the lights initially come on in response to hunger, and again just prior to lights off in anticipation of the dark period (May and Lott, 1994).

Previous data from this work have shown that daylength has a number of impacts on productivity and welfare in commercial broilers. Adding darkness to a photoperiod program can result in equal or even improved growth rate and improves the efficiency of feed conversion (Schwean-Lardner et al., 2011). It also results in expression of many behaviours that appear to be eliminated or greatly reduced when near-constant photoperiods are used, and birds are much less alert or responsive (Schwean-Lardner thesis, Chapter 4) . In both of these cases, the authors have hypothesized that one of the factors resulting in these changes is that birds exposed to near-constant photoperiods are sleep-deprived and this condition can at least partially explain these effects. However, the exact mechanism whereby sleep deprivation occurs has not been established. It has been shown that birds can sleep in the light period and it has been speculated that the quantity and quality of sleep may suffer under near-constant photoperiods (Ayala-Guerrero et al., 2003). If a bird attempts to sleep during the day period, but other birds are active at the same time (hence a lack of flock behavioural rhythms), the sleep will be repeatedly disrupted. This has been termed sleep fragmentation (Bonnet, 2005) and the impact of such deprivation over a longer period of time has been noted to be similar to total sleep deprivation in humans (Bonnet, 1986). The end result of sleep fragmentation can be a reduction in both quantity and quality of sleep.

The objective of this study was to determine if a flock of birds develops a pattern of behavioural expression during the photophase and in its physiological functioning (via monitoring melatonin production) under lighting programs with four different daylengths. If rhythms can develop under all lighting programs, then it could be assumed that disruption to sleep patterns would not occur, making sleep deprivation less likely. However, if birds under particular lighting programs do not develop behavioural or physiological rhythms, then sleep is likely disrupted, leading to sleep deprivation and poor welfare.

MATERIALS AND METHODS

All experimental work was carried out with permission of the University of Saskatchewan Animal Care Committee. All procedures followed the recommendations of the Canadian Council of Animal Care (1993) as listed in the Guide to the Care and Use of Experimental Animals.

The work discussed in this paper was part of a larger experiment, which involved examining the impact of daylength, bird genotype and bird gender on productivity traits in commercial broilers, and of daylength on behavioural expression of those broilers. These experiments have been described in detail in previous articles (Schwean-Lardner et al., 2011).

For this work, the impact of daylength on the rhythm of behavioural expression and melatonin production was examined in male Ross x Ross 308 broilers. A total of four lighting programs were tested, with graded levels of darkness. These lighting programs were chosen specifically to mimic a practical range of programs used in commercial production units. The tested programs were 14 hours (h) of light (L) and 10 h of dark (D) (14L:10D or 14L), 17L:7D (17L), 20L:4D (20L) and 23L:1D (23L), and were initiated at 7 days (d) of age. In all cases, light was introduced at 06:00 h. The scotoperiod of each program was provided in one section within each day, with a light intensity of near 0. The light intensity of the photoperiods was 10 lux. Prior to program initiation (d7), all birds were maintained on 23L:1D at 20 lux intensity. Light was provided by incandescent bulbs.

An infrared camera system was mounted on the ceiling of a pen (2.3 x 2 m) of Ross x Ross 308 male broilers (n=53 at placement) to allow continuous capture of behavioural actions over a 24-h period, with the camera capable of capturing all areas within the pen. One pen per lighting treatment was recorded on the first day of test, and a second replicate pen per treatment was recorded on the following day.

Data were collected in two separate experiments. Video recordings were done at 27 and 28 d (27 d) of age in the first experiment, and at 42 and 43 d (42 d) in the second.

Data collection

After video recordings were complete, behaviour was observed using the Genetec Omnicast Live Viewer 3.5 system. The number of birds performing specific behaviours was counted at 10 min scan intervals for the full 24 h per replication (24 x six measurements per day). To allow this to happen, the video frame for each time interval was stopped and restarted until all birds were classified into one of the predetermined categories. Behavioural categories included inactive resting (lying on the straw not performing any other behaviours), walking, standing (standing and not performing any other behaviour), preening (manipulating feathers on own body, either lying or standing), leg or wing stretching (standing or lying down), dustbathing, pecking or foraging at the litter, being present at the feeder (feeding; at the feeder with head into the lip of the feeder), and being present at the drinker (drinking standing with head directly under the drinker line). It was not possible to determine if inactively resting birds were sleeping. Data were separated into photophase and scotophase periods, and photophase behavioural rhythms were used for this work. The data were expressed as percent of time performing the various behaviours.

At 21 d of age, blood samples were collected throughout a 24-h period from three birds per room and two rooms per lighting treatment (N = 6 per treatment per time), eight times for birds raised on 14, 17 and 20 h light and six times for birds raised on 23L, in which only one bleed was possible during the short scotoperiod. Different birds were sampled each time and blood samples were collected via decapitation. This allowed the determination of synchrony on a flock basis rather than an individual basis. During the dark period, a technician entered the production rooms in the dark, quietly removed one bird at a time and walked approximately 3 m

to a separate dark room. Lights were turned on only immediately prior to decapitation to remove the possibility of light impacting melatonin levels. Sampling times were chosen to include one bleed during the mid-scotoperiod and one mid-photoperiod, then equally on each side of that time depending on the daylength (Table 1). Blood was centrifuged and serum stored at -20° C until testing. Serum samples were analyzed for melatonin (ng/mL) using a radioimmunoassay kit¹ to determine the amount of melatonin present. Intra- and inter-assay coefficient of variations (CV) for the assays were 7.23 and 8.85 respectively.

Statistical analyses

To test for the presence of behavioural rhythms during the photoperiod, each behavioural category within a lighting treatment was tested for trend analysis using regression analysis (Proc Reg and Proc RSReg of SAS). Behavioural data (expressed as a percent of time) were (log+1) transformed prior to analysis for determination of significance in relationships between behaviour and time during the photophase. A lack of relationship during the photophase indicated no behavioural rhythm.

Serum melatonin data were analyzed by two methods. The minimum and maximum levels were analyzed as a completely randomized design using ANOVA analysis of SAS, with lighting program as the main effect. Regression analysis was used to determine circadian rhythm development in the flock (Proc Reg and Proc RSReg of SAS). Melatonin rhythms were tested using the entire photoperiod-scotoperiod data set.

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RESULTS

Behaviour

Inactive resting

At 27 d, significant photophase quadratic relationships were found between the behaviour and time for flocks raised on 14L and 17L (Figures 1a, b). In both of these cases, trends in the data show that inactive resting drops immediately after lights come on, and again to a lesser degree prior to lights off, the latter likely due to anticipatory feeding. Anticipatory feeding is not seen in 20L (Figure 1c) or 23L flocks (Figure 1d); 23L birds are inactive for most of the day period. At 42 d, similar quadratic rhythms are noted under 14L and 20L, with a linear increasing relationship within the day for flocks raised under 17L (Figures 10a, b, c). No relationship is found with flocks on 23L (Figure 10 d).

It is notable that level of inactive resting at the beginning of the photoperiod is impacted by daylength (Figure 1a, b, c and d). The proportion of inactive birds is less than 2% in birds raised on 14L, less than 25% for 17L, 40% for birds raised on 20L and just under 75% for birds raised on 23L. A similar trend can also be seen in the 42 d flock (Figures 10a, b, c, d).

Walking

The percentage of flock walking was quadratically related to time of the day period for flocks on 14L and 17L at 27 d (Figures 2a, b). Under 14L, the behaviour was highest at the end of the day, however standing and feeding peaks were found early in the day, suggesting birds might have moved to the feeder at a quicker rate. Under 17L, the behaviour was higher early and late in the day. No rhythms were found for 20L (Figure 2c) and 23L (Figure 2d) birds. At 42 d, a linear relationship was noted in flocks under 14L (Fig. 11a) with highest walking early in the day. No other treatments resulted in rhythms during the day period (Figures 11b, c and d).

Standing

Standing during the photoperiod occurred in a quadratic fashion for flocks under 14L and 17L at 27 d (Figures 3a, b) and for flocks under 14L, 17L at 42 d (Figures 12a, b). In these flocks, standing occurred at the highest peak immediately post lights on, and a small increase was noted prior to lights off. A linear decrease in standing is noted for flocks under 20L at 42 d (Figure 12c). No rhythm was noted at 27 d under 20L or 23L (Figures 3c, d), or under 23L at 42 d (Figure 12d).

Preening

Flocks under 14L and 17L demonstrate a quadratic rhythm in preening during the day at 27 d (Figure 4a, b), but the nature of the quadratic response differs. Birds begin preening approximately 2 h after lights are on at 27 d, and then continue to preen throughout the day. Under 17L (Figure 4b), preening begins and is at its highest level just after lights come on. No statistically discernible rhythms were noted at 27 d (Figures 4c, d) or 42 d (Figures 13a, b, c, d). For the older age, preening appeared to occur sporadically with no noticeable rhythm.

Stretching

On 27 d, flocks under 14L (Figure 5a) showed a significant quadratic relationship in stretching throughout the day period, with the highest value occurring late morning to mid-afternoon. No rhythms were statistically identified for birds under other photoperiod regimes (Figures 5b, c, d). A similar situation occurs at 42 d (Figures 14a, b, c, d). A small portion of the 14L flock demonstrated stretching behaviour during the scotoperiod, and this may suggest that a 10 h dark period is more than adequate to obtain appropriate sleep.

Feeding

Flocks on 14L (Figure 6a) and 17L (Figure 6b) showed quadratic relationships between feeding and time of the day period at 27 d, as did the 14L flock at 42 d (Figure 15a). In these cases, flocks were at feeders at the highest level immediately after lights come on and just prior

to lights turning off. This behavioural rhythm likely is responsible for the increases in walking and standing at the same time periods. Only flocks raised on 14L (Figure 6a, 15a) at both ages showed a small level of night time feeding in this work.

Drinking

Drinking patterns are similar to those for feeding, with drinking activity highest early in morning and just before lights going off in flocks raised on 14, 17 and 20L at 27 d (Figures 7a, b, c) and 14L at 42 d (Figure 16a). No other rhythms were found (Figures 7d and 16b, c, d).

Litter pecking

Litter pecking followed a quadratic rhythm with time of day in 14L flocks at 27 d (Figure 8a) and in flocks under 14 and 17L at 42 d (Figure 17b), with less litter pecking early and late in the day.

Dustbathing

In 27 d of age flocks under 14, 17 and 20L (Figures 9a, b, c) and 42 d of age flocks under 14L and 17L (Figures 18a, b), dustbathing was performed in a quadratic relationship over time during the day period with the highest expression of behaviour near mid-day. Regression analysis determined a linear relationship with the incidence of dustbathing increasing during the day for birds from the 23L treatment at 27 d (Figure 9d). Dustbathing occurred infrequently at 42 d under 20L (Figure 18c), and ceased to occur under 23L (Figure 18d).

Melatonin

Significant differences were found in the daily minimum and maximum level of melatonin produced (Table 2). The lowest values were measured in birds from flocks on 14L and 17L, and the highest peak was noted from birds raised on 20L. When calculating the range between the lowest point of the valley and the highest point of the peak, the largest amplitude was noted with birds raised on 14L and 20L, and the lowest from the 23L flock. Figure 19 (a, b, c and d) shows

the melatonin levels as collected throughout the day. Rhythms (as defined by significant quadratic regression analysis) were found for flocks on the 14L, 17L and 20L treatments over a 24-h period, but not for the 23L flock.

DISCUSSION

Daylength has a clear impact on patterns of behavioural expression in broiler chickens during the photophase. While flocks raised on 14L and 17L form rhythms in virtually all behaviours measured, those on 20 h of light only exhibit flock patterns for some behaviours, suggesting that the need for darkness based on rhythm development for broiler flocks lies somewhere between 4 h and 7 h of darkness per day. Flocks on near-constant light (23L) do not form flock-based rhythms during the day period, suggesting sporadic performance of behaviours and low levels or elimination of behaviours. Therefore, the data indicate that the use of near-constant light results in the disruption of behavioural rhythms for broiler flocks, and that 4 h of darkness may not be enough to ensure full rhythm development. One possible reason for why this occurs is that long daylengths cause sleep deprivation. Sleep deprivation can negatively impact animal welfare (Blokhus, 1983), so determining a bird's ability to sleep should be considered when well-being is assessed.

Although there is still a lack of understanding of the entire function of sleep, it is known that sleep is vital for animals (Saito et al., 2003). This is also true for poultry where sleep contributes a positive function in energy conservation, tissue regeneration and an ability to adapt to stress (Blokhus, 1983).

Aspects of sleep differ between species, but mammals and birds appear to be quite similar. Sleep has two primary stages – slow wave sleep (SWS) and fast wave sleep (FWS) (Blokhus, 1983), the latter also being termed rapid eye movement (REM) (Rattenborg et al., 2009). These phases can be separated via electroencephalography (EEG) (Rattenborg et al.,

2009), but not without difficulty (van Luitelaar et al., 1987). It is established that the phase of sleep changes throughout the sleep period, and in laying hens it has been noted that the very early stages of sleep are characterized by more SWS, which lessens throughout the night (van Luitelaar et al., 1987). A relevant finding is that FWS sleep never occurs without SWS as a precursor (van Luitelaar et al., 1987; Ayala-Guerrero et al., 2003).

Deprivation can have many forms, and can impact both the quality and quantity of sleep with similar effects. Without all stages of sleep occurring in each episode, the quality of sleep can be negatively affected because different restorative functions occur during different phases. FWS appears to have a more important function in stress adaptability than does SWS (Blokhus, 1983). While memory is impacted by sleep in general, each stage is important for different reasons. Born et al. (2006) found that both SWS and FWS are important for different types of memory and speculated that it was likely through the production of different neurotransmitters and neurohormones in each of the sleep phases. For example, serotonin production is associated with slow-wave sleep (Sabelli and Giardina, 1970).

A specific type of sleep deprivation, termed sleep fragmentation (Bonnet, 2005), occurs when an animal is woken repeatedly during the sleep period. Sleep fragmentation appears to disrupt the normal sleep phase routine (Bonnet, 2005), which as described above reduces sleep quality. Even after one night of sleep fragmentation, humans' demonstrated poor psychomotor performance, poor reactivity, and increased sleepiness. After 64 h of such repeated arousals, the human response mimicked total sleep deprivation (Bonnet, 2005).

Melatonin production has a strong circadian rhythm with high production during the dark period and low production during the day period. Melatonin is an important neurohormone involved in many physiological functions, is known to be involved in modulating sleep, and has been shown to induce daytime SWS in pigeons (Phillips and Berger, 1992). It plays a major

role in the development of the bird circadian clock, and elimination of the melatonin production can result in a disruption of circadian rhythms (Gwinner et al., 1997). While sleep may occur during the light period, quality is reduced (Manser, 1996; Rattenborg et al., 2005), and it is speculated that this is attributed to the suppression of melatonin synthesis (Rattenborg et al., 2005). Other studies have reported that melatonin production is minimal during constant light (Apeldoorn et al., 1999).

One of the important aspects of circadian rhythm development is to allow flocks to perform behaviours at similar times (Kohsaka and Bass, 2006). Because the development of flock behavioural rhythms in this work was not found when a near-constant light program was used, it can be assumed that portions of the flock attempted to sleep while other birds were expressing other behaviours. This would result in the disruption and constant awakening of sleeping birds, and sleep fragmentation seems likely. In turn, fragmentation of sleep would eliminate the achievement of normal night time sleep phases that are essential for health and well-being (Chen and Kushida, 2005). Counter to this conclusion is research with turkeys where birds exposed to constant light did reach REM sleep for a period of time (Ayala-Guerrero et al., 2003). However, it is important to note that the birds were tested singly in a test chamber, so interruption by other birds would not have occurred. Therefore, it may not be predictive of practical poultry production conditions that are more similar to the conditions used in the present research. Based on the degree of behavioural asynchrony, the above evidence of sleep deprivation is particularly true of the flocks raised on near-constant light, but there is also evidence to a lesser extent that this situation also exists for the 20L birds.

The lack of a flock melatonin circadian rhythm for birds given the 23L treatment demonstrates a lack of synchrony within the flock and is in agreement with the lack of daily behavioural patterns. Interestingly, mean melatonin values for this treatment were not that

different than for other lighting treatments where a clear circadian rhythm was present. This is different than previous results where birds produced low levels of melatonin under constant light conditions (Apeldoorn et al., 1999). One explanation for this difference could be that one hour of darkness was sufficient to result in increased melatonin synthesis even if patterns of melatonin synthesis were not coordinated. It needs to be recognized that circadian melatonin rhythms in this study were not obtained from repeat bleeding of the same bird but rather from bleeding each bird only once at a specific time.

Establishing the welfare status of an animal is difficult and must rely on a variety of assessment parameters. It is well recognized that behavioural assessment is one of the strongest indicators of animal welfare (Duncan, 2005). Combining the welfare implications of behaviour assessment with the presence of rhythms in expression of specific behaviours or melatonin production is specifically relevant to this paper, as Bessei (2006) has stated that the development of circadian rhythms is important for welfare to be optimal. Our research has demonstrated that long daylength negatively affects behavioural expression (Schwean-Lardner thesis, Chapter 4) and this research further demonstrates that daily behavioural patterns and physiological rhythms are also lacking. Other research has similarly indicated that continuous light eliminates some circadian patterns in behaviour. Locomotory behaviours, and activity in general, cease to follow a rhythm with the use of constant light (Reiter and Bessei, 2002). The behavioural and melatonin data from this paper cannot demonstrate that individual bird circadian rhythms have been eliminated, as single birds were not followed throughout for either parameter. However, they do show that, at the very least, the rhythms of behaviour and melatonin production have become desynchronized in the flock; hence, on a group basis, specific behaviours occur sporadically with no rhythm. As a consequence, sleeping likely cannot occur without constant interruptions, which leads to sleep fragmentation as described above.

Data from this research suggest that 4 h of darkness is sufficient to synchronize melatonin synthesis; however, behavioural rhythms are very limited and are not as frequent as seen with 7 h or more of darkness. One h of darkness appears to be a weak or ineffective zeitgeber for flock synchronization.

While virtually no active night time behaviour occurred in flocks under 17, 20 or 23L, activity was noted in birds under 10 h of darkness. Birds from this treatment showed night time feeding. This suggests that a 10 h dark period is too long with regards to appetite. It may also suggest, as stated above, that 10 h of darkness provides more than enough time for the birds to achieve their required sleep period. Night time feeding is not common, unless circumstances, such as long dark periods, lead the birds to this activity (Savory, 1976). Birds from this treatment also showed stretching activity and standing activity during the dark period, likely associated with the night time feeding and adequate sleep. These data suggest that a 10 h dark period is more than required for the well-being of birds.

To conclude, exposing birds to 4 or more h of darkness allows the development of flock photophase behavioural rhythms and melatonin circadian rhythms, but longer daylengths do not permit uniform development of these characteristics. Shorter daylengths (14 and 17L) increase the expression of behavioural rhythms as well as change the amplitude characteristics of melatonin rhythms. The results also support the hypothesis that birds reared under constant or near-constant light are sleep deprived. The combination of reduced or eliminated circadian and daily rhythms, evidence of sleep deprivation and previous reports of other aspects of bird welfare assessment (Schwean-Lardner thesis, Chapter 4; Schwean-Lardner et al., 2011) support the recommendation that continuous or near-continuous light not be used for broiler production on welfare grounds.

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TABLE 5.1 Serum collection times for melatonin analysis. * indicates time of each collection

	A.M.					P.M.					A.M.														
	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	
14L		*						*					*				*		*	*		*	*	*	*
17L		*	*				*								*	*	*		*	*		*	*	*	*
20L		*			*					*							*		*	*		*	*	*	*
23L		*					*					*					*		*	*		*	*	*	*

Shaded area indicates dark period

TABLE 5.2. The impact of daylength on minimum and maximum daily melatonin levels (pg/mL) and the range between minimum and maximum values

	Daylength (h)				SEM
	14	17	20	23	
Minimum	29.5 ^C	39.8 ^{BC}	51.2 ^{AB}	53.2 ^A	2.80
Maximum	119.7 ^B	112.3 ^B	149.5 ^A	98.0 ^B	5.69
Range	90.2 ^A	72.5 ^{AB}	98.3 ^A	44.9 ^B	6.35

^{A,B,C} Means within a row lacking a common superscript vary significantly ($P \leq 0.05$).

SEM: standard error of the mean.

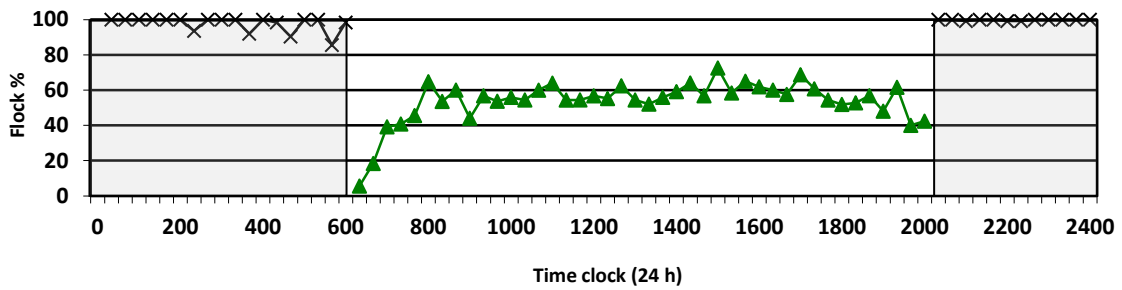


FIGURE 5.1a. Percent of flock inactive resting of birds on 14L:10D at 27 d. The black checked line represents the scotophase period, and the green triangle line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

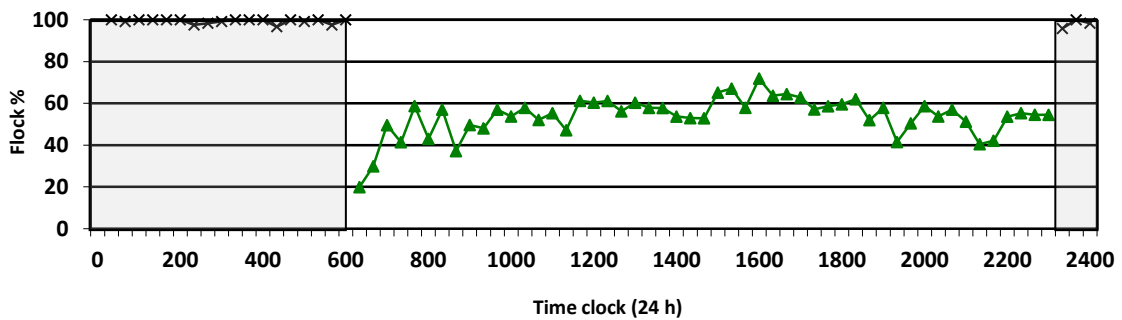


FIGURE 5.1b. Percent of flock inactive resting of birds on 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically over time during the photophase ($P=0.0001$).

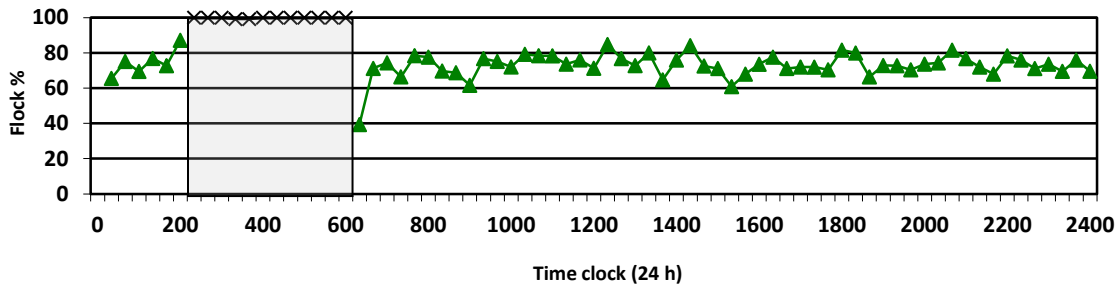


FIGURE 5.1c. Percent of flock inactive resting of birds on 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

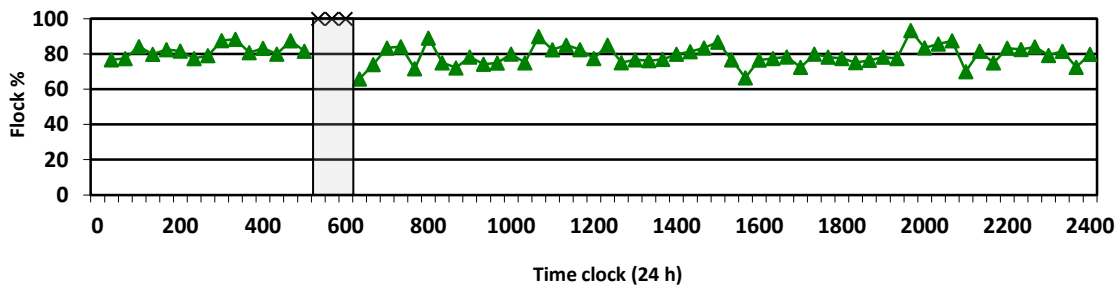


FIGURE 5.1d. Percent of flock inactive resting of birds on 23L:1D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

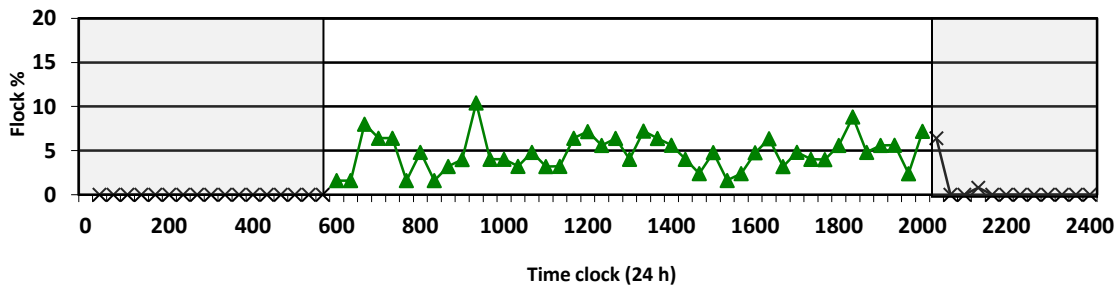


FIGURE 5.2a. Percent of flock walking of birds on 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0001$).

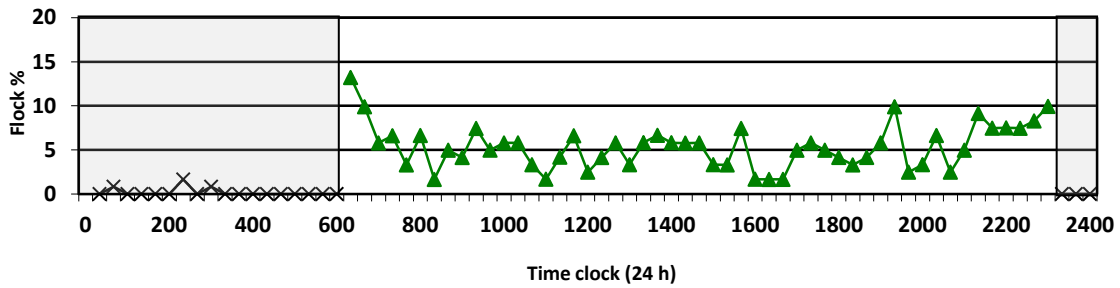


FIGURE 5.2b. Percent of flock walking of birds on 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0003$).

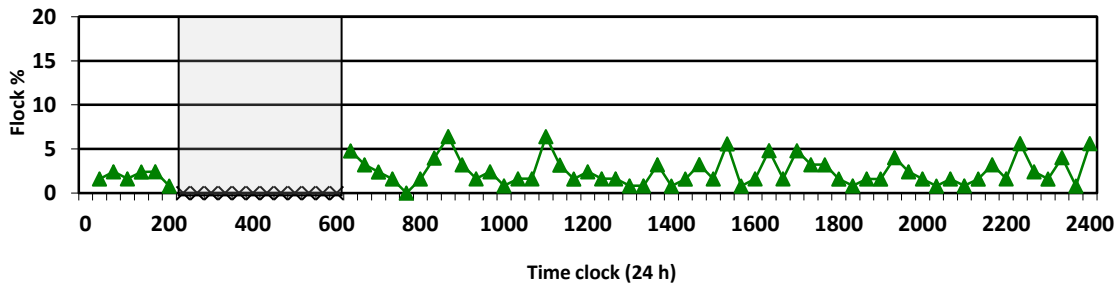


FIGURE 5.2c. Percent of flock walking for birds on 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

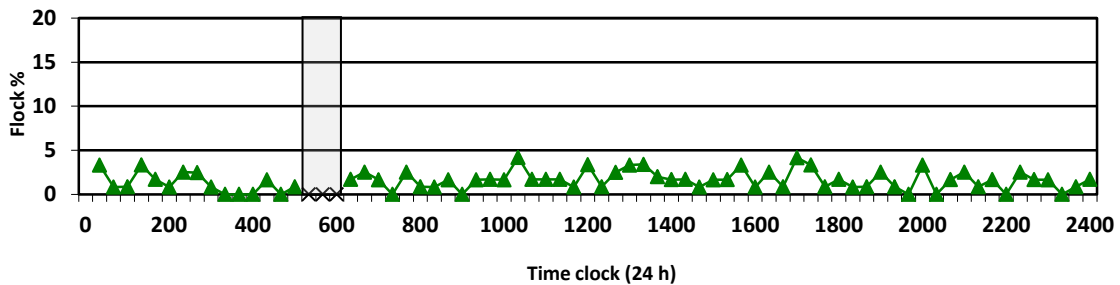


FIGURE 5.2d. Percent of flock walking for birds on 23L:1D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

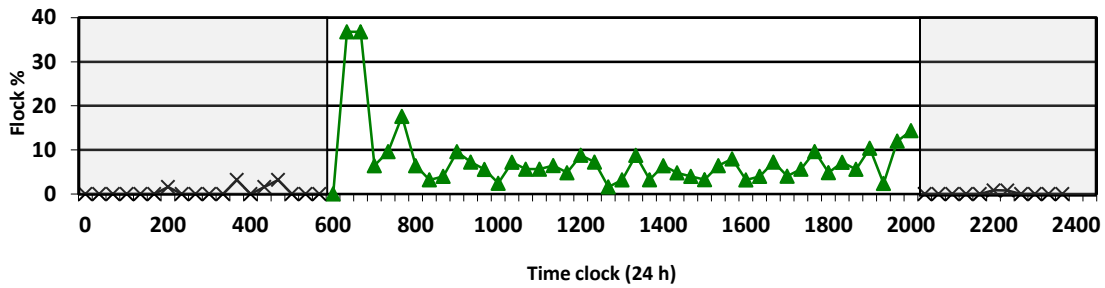


FIGURE 5.3a. Percent of flock standing for birds on 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

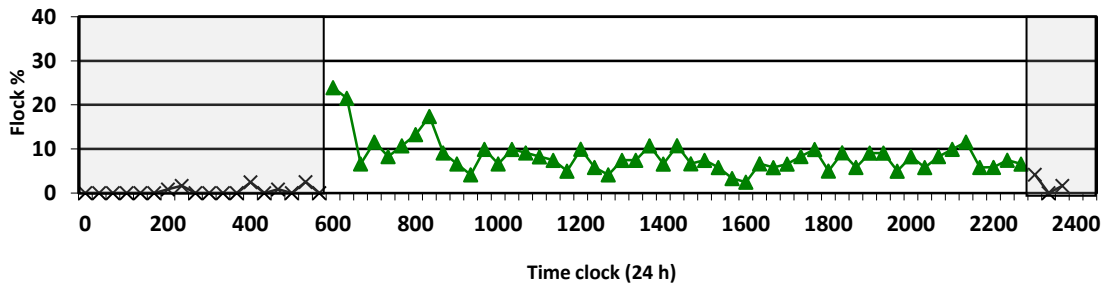


FIGURE 5.3b. Percent of flock standing for birds on 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0007$).

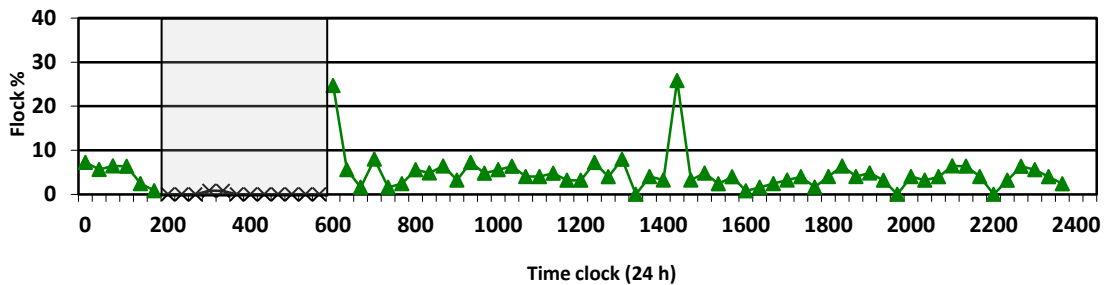


FIGURE 5.3c. Percent of flock standing for birds on 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

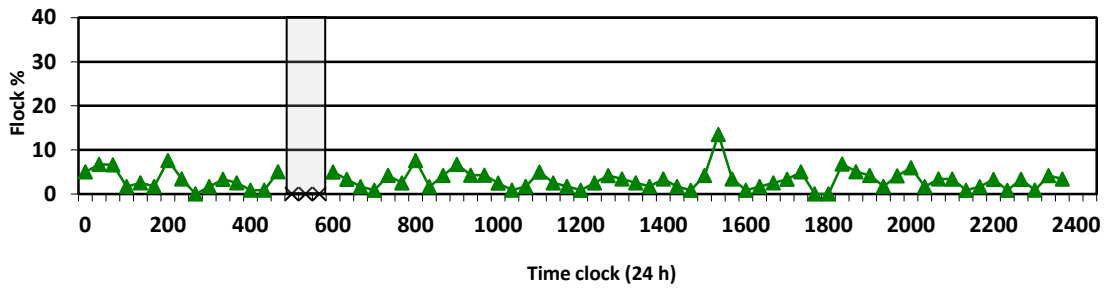


FIGURE 5.3d. Percent of flock standing for birds on 23L:1D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

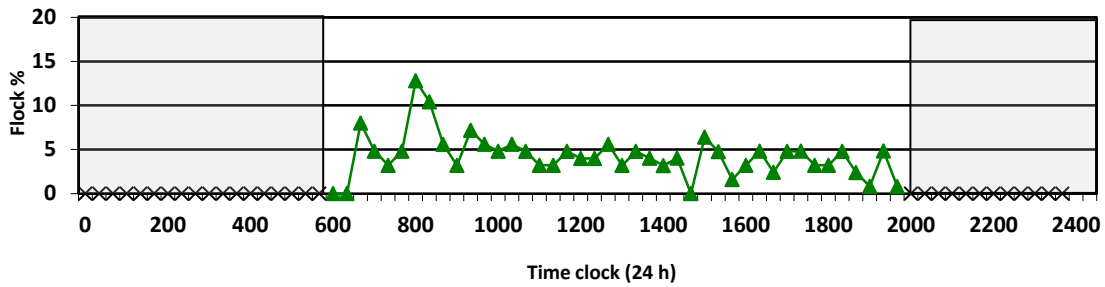


FIGURE 5.4a. Percent of flock preening for birds on 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0001$).

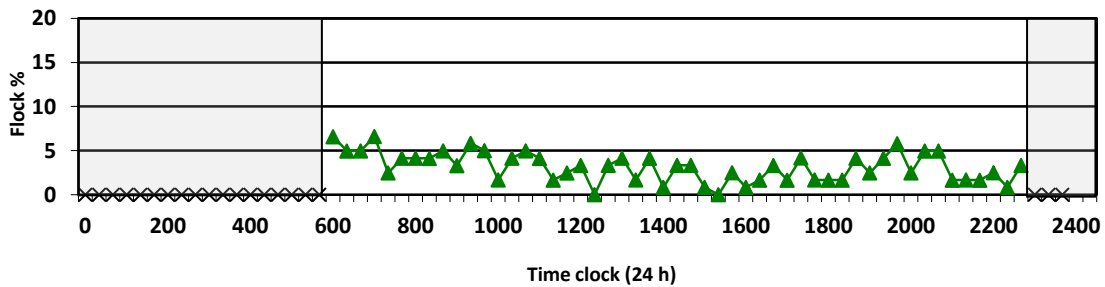


FIGURE 5.4b. Percent of flock preening for birds on 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0089$).

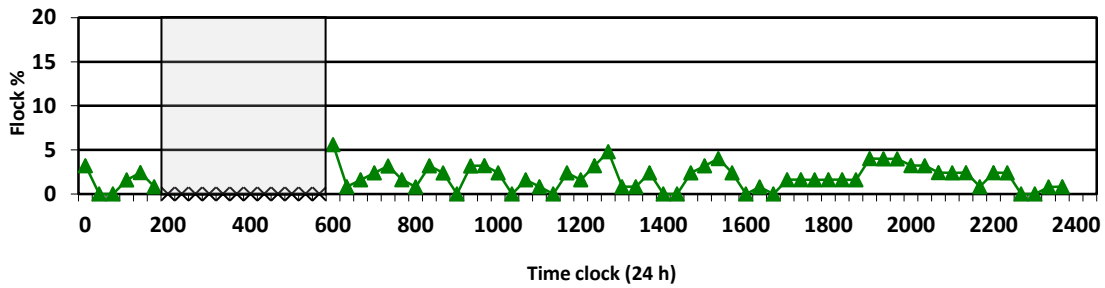


FIGURE 5.4c. Percent of flock preening for birds on 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

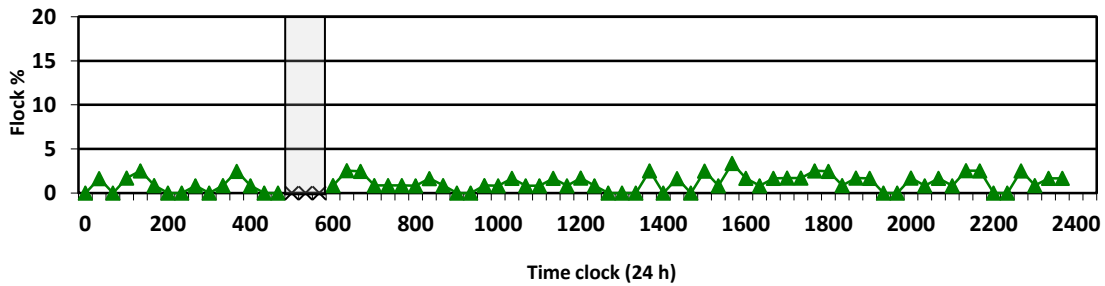


FIGURE 5.4d. Percent of flock preening for birds on 23L:1D. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

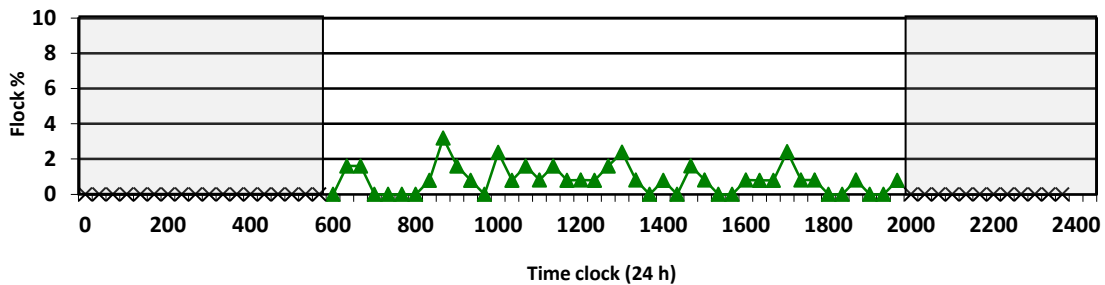


FIGURE 5.5a. Percent of flock stretching (wing or leg) under 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant ($P=0.0001$).

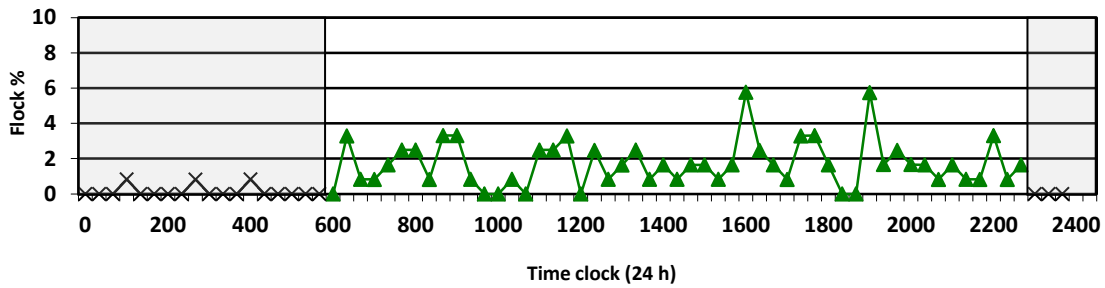


FIGURE 5.5b. Percent of flock stretching (wing or leg) under 17L:7D at d 27. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

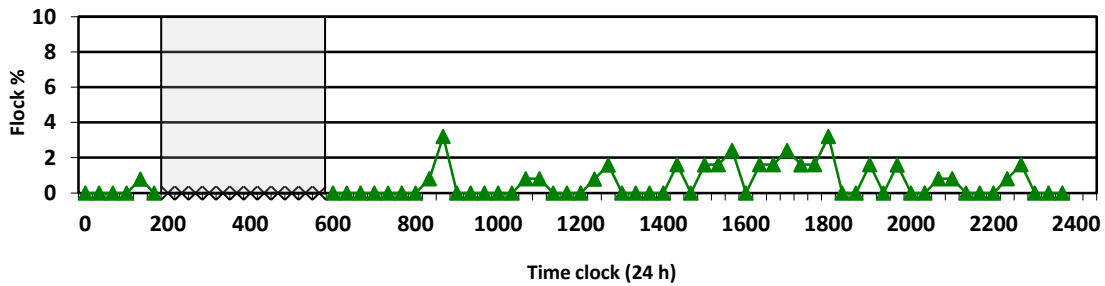


FIGURE 5.5c. Percent of flock stretching (wing or leg) under 20L:4D at d 27. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

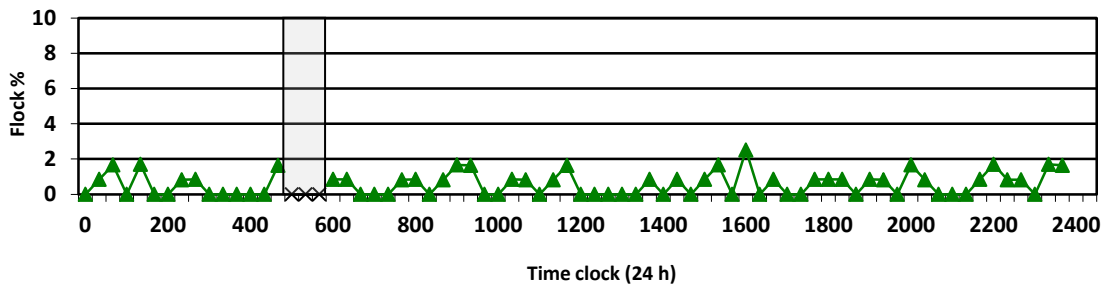


FIGURE 5.5d. Percent of flock stretching (wing or leg) under 23L:1D at d 27. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

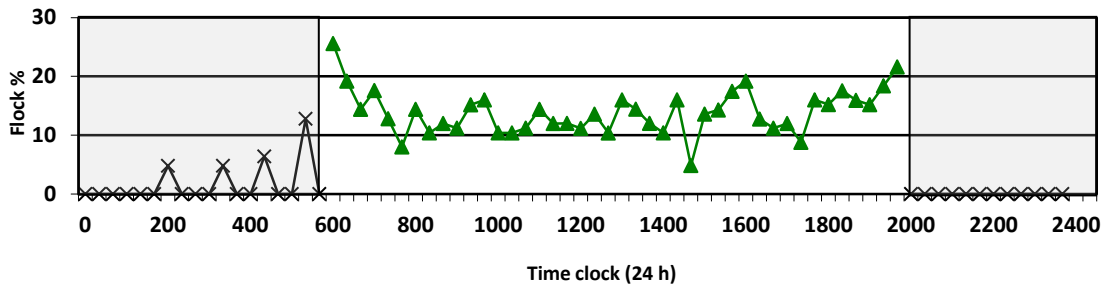


FIGURE 5.6a. Percent of flock at the feeder under 14L:10D at d 27. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

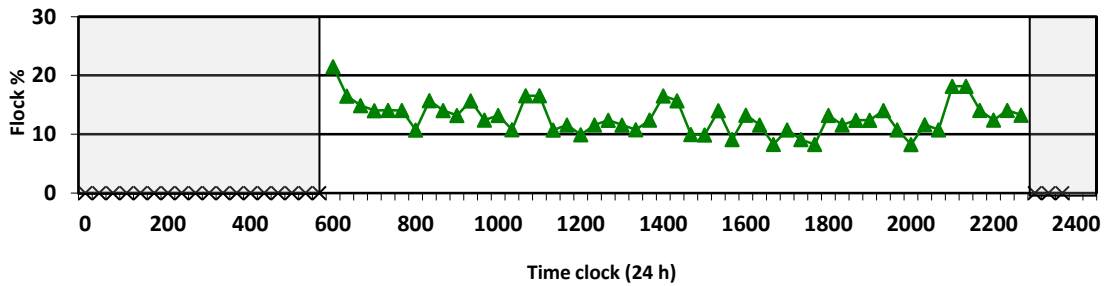


FIGURE 5.6b. Percent of flock at the feeder under 17L:7D at d 27. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0004$).

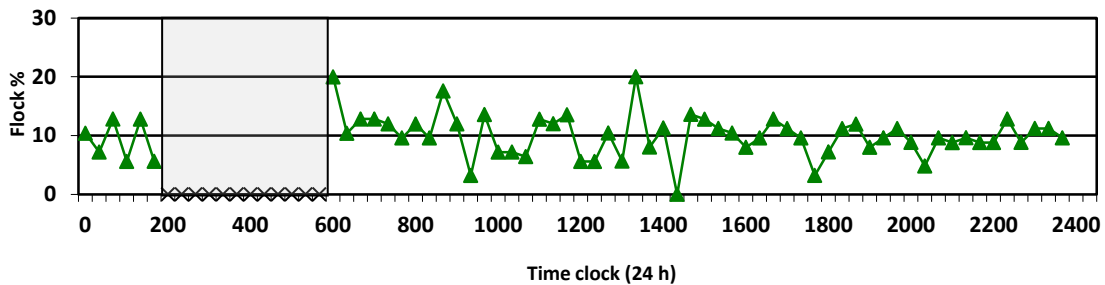


FIGURE 5.6c. Percent of flock at the feeder under 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

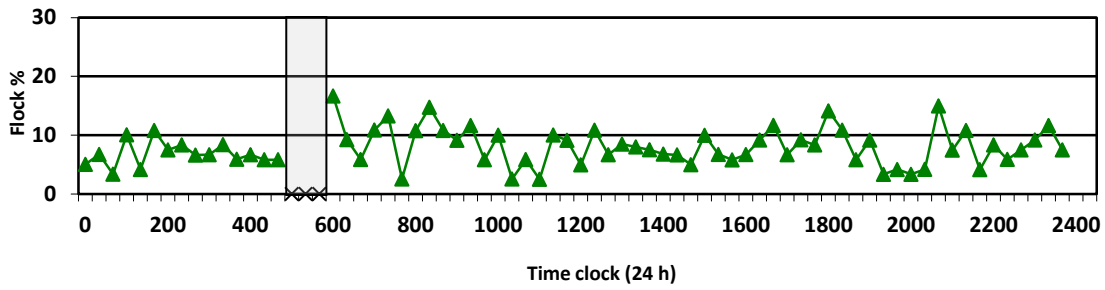


FIGURE 5.6d. Percent of flock at the feeder under 23L:1D at d 27. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

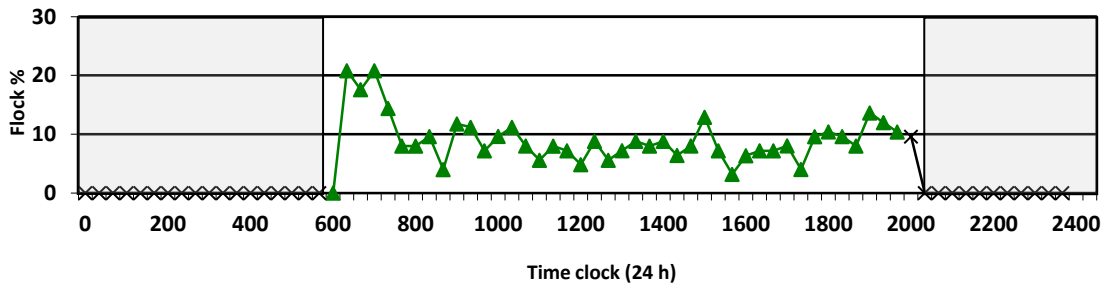


FIGURE 5.7a. Percent of flock at the drinker under 14L:10D at d 27. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

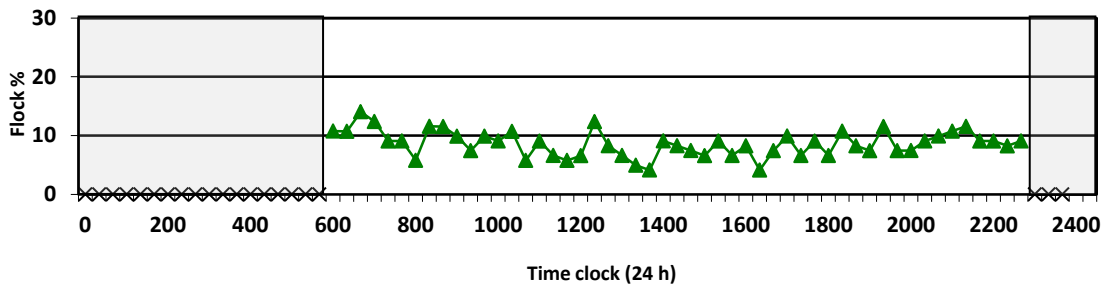


FIGURE 5.7b. Percent of flock at the drinker under 17L:7D at d 27. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

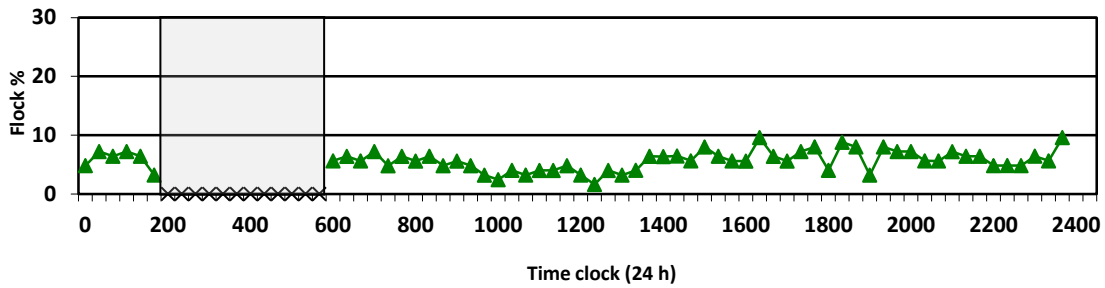


FIGURE 5.7c. Percent of flock at the drinker under 20L:4D at d 27. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0283$).

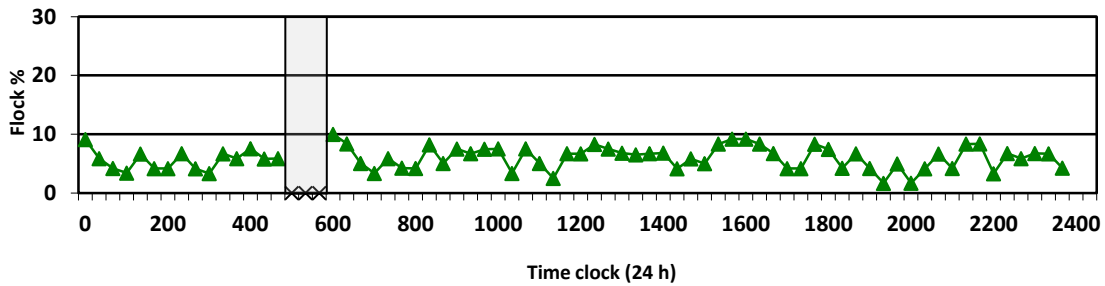


FIGURE 5.7d. Percent of flock at the drinker under 23L:1D at d 27. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

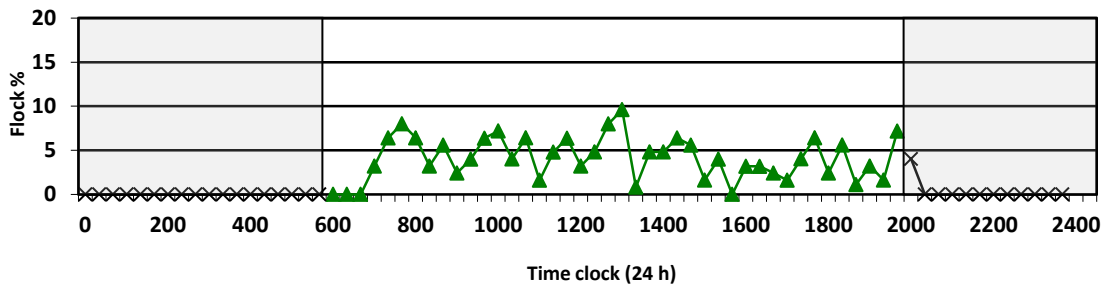


FIGURE 5.8a. Percent of flock litter pecking under 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

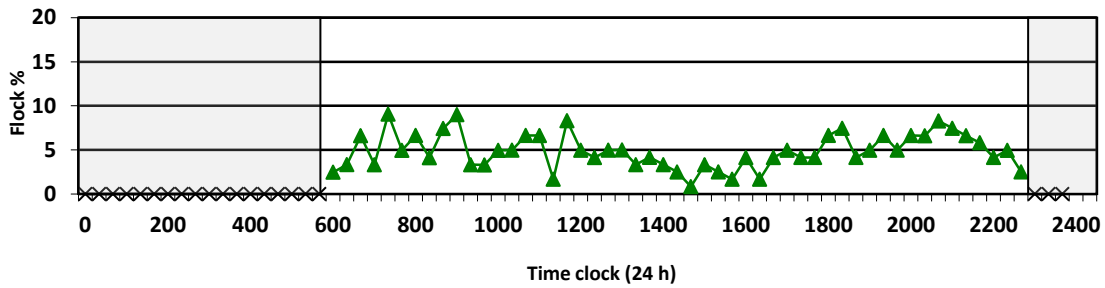


FIGURE 5.8b. Percent of flock litter pecking under 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

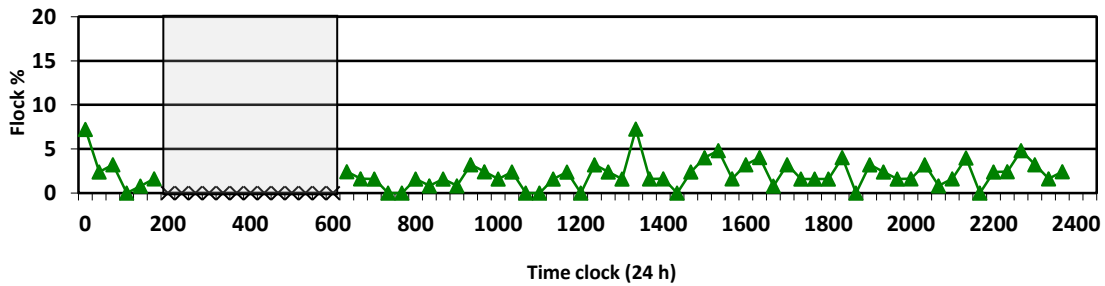


FIGURE 5.8c. Percent of flock litter pecking under 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

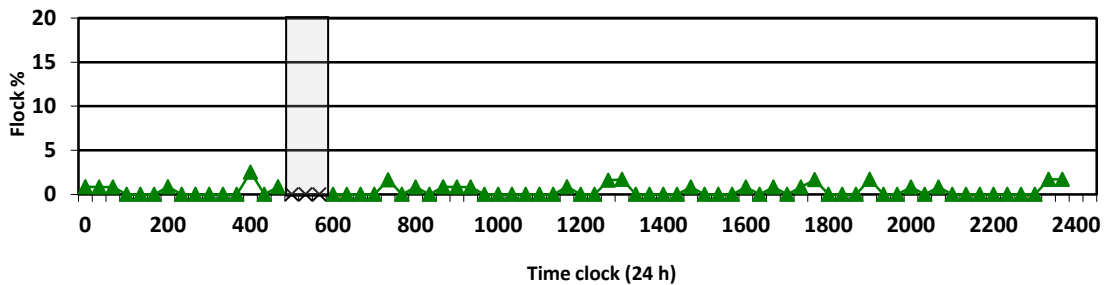


FIGURE 5.8d. Percent of flock litter pecking under 23L:1D at 27 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

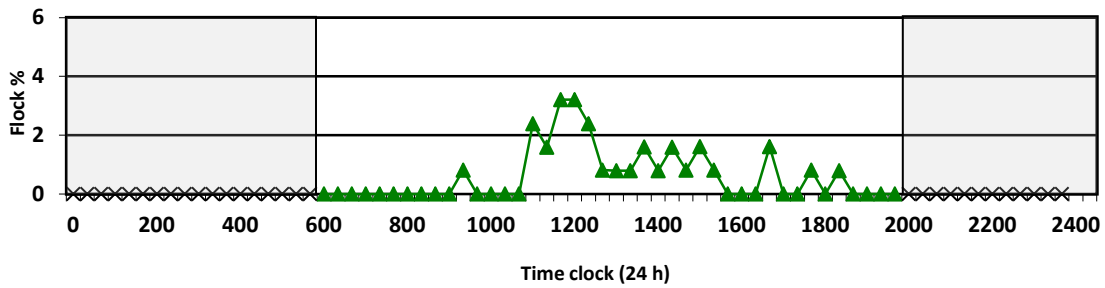


FIGURE 5.9a. Percent of flock dustbathing under 14L:10D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0003$).

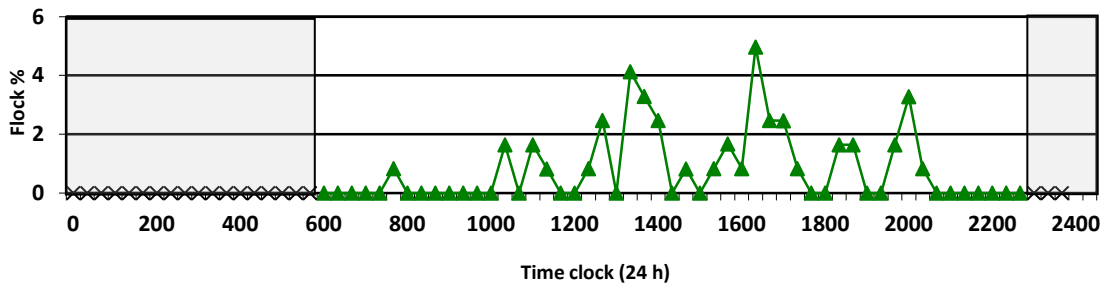


FIGURE 5.9b. Percent of flock dustbathing under 17L:7D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

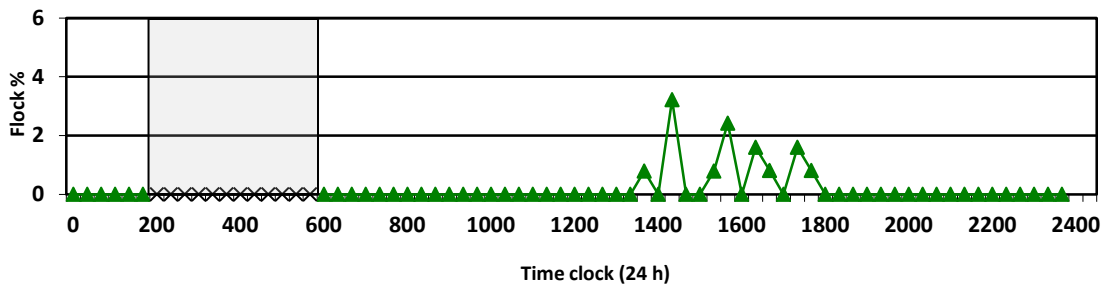


FIGURE 5.9c. Percent of flock dustbathing under 20L:4D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0258$).

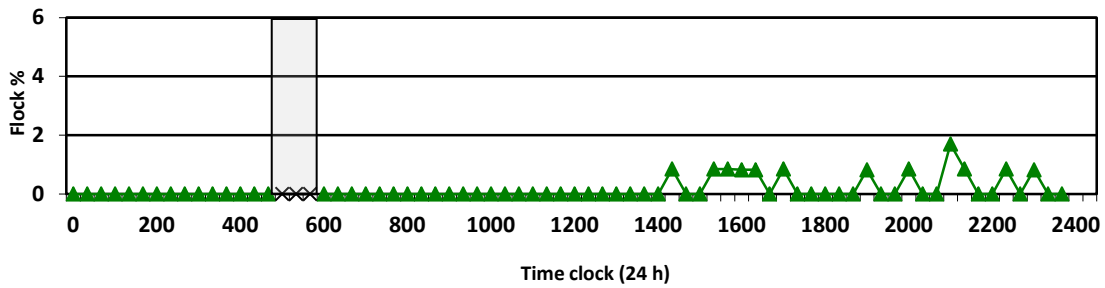


FIGURE 5.9d. Percent of flock dustbathing under 23L:1D at 27 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0008$).

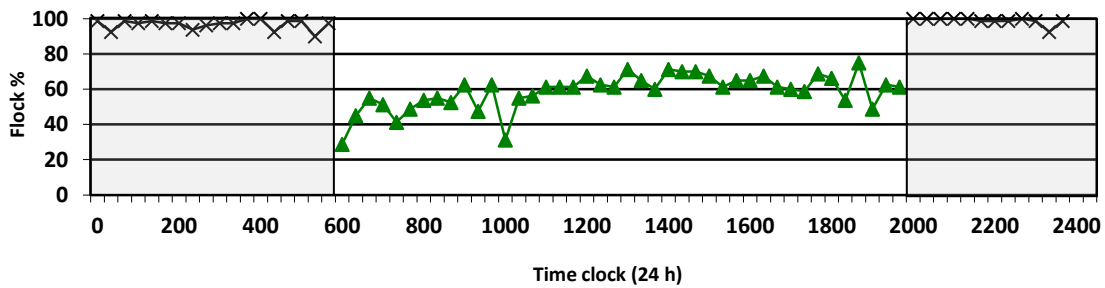


FIGURE 5.10a. Percent of flock inactive resting under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0002$).

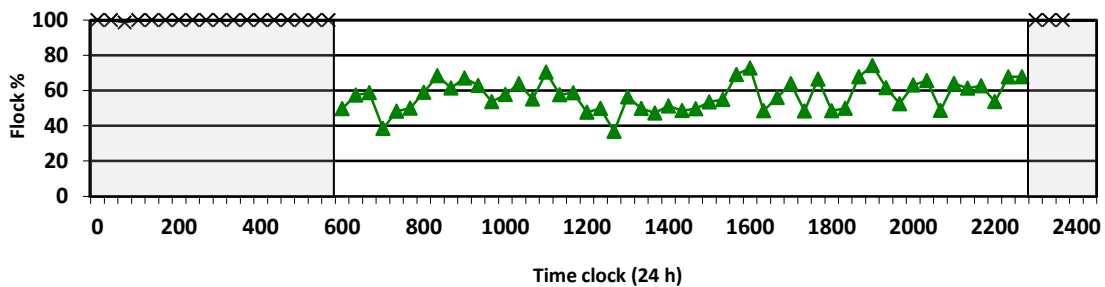


FIGURE 5.10b. Percent of flock inactive resting under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0406$).

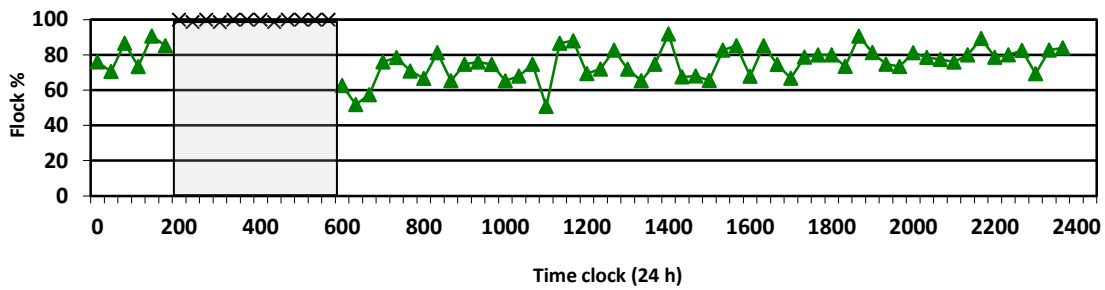


FIGURE 5.10c. Percent of flock inactive resting under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0050$).

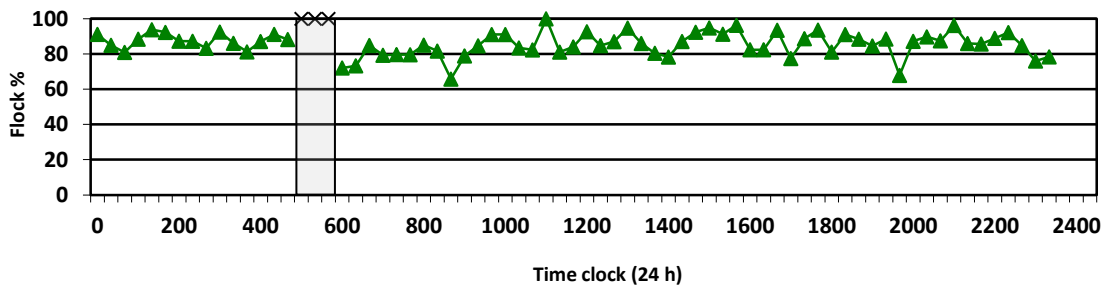


FIGURE 5.10d. Percent of flock inactive resting under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

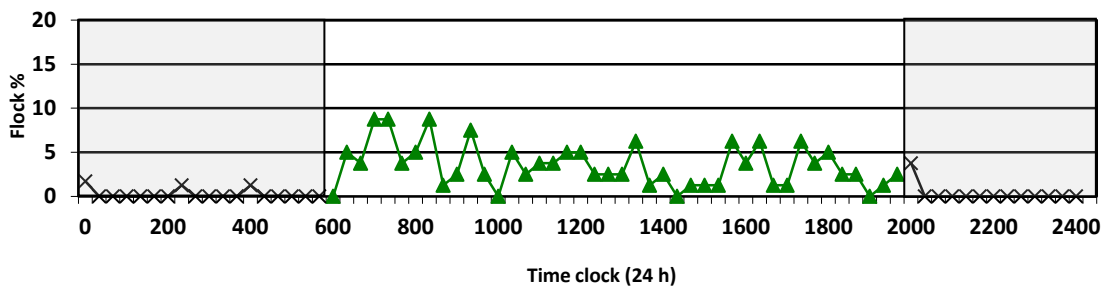


FIGURE 5.11a. Percent of flock walking under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0164$).

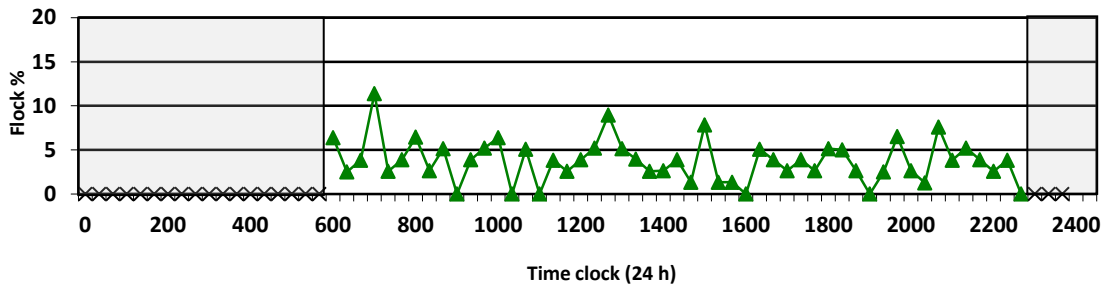


FIGURE 5.11b. Percent of flock walking under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

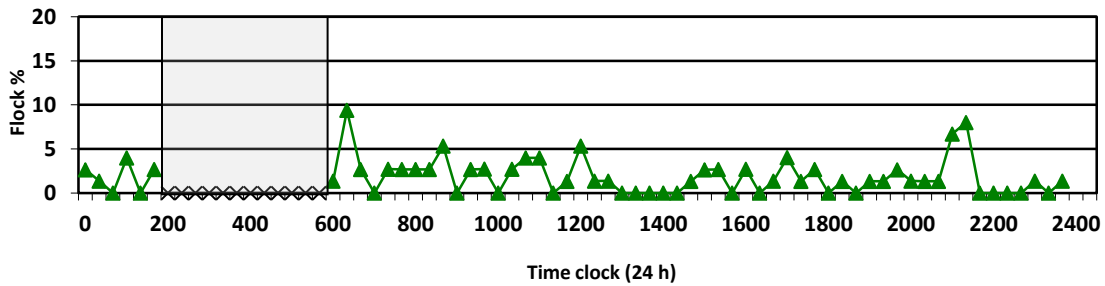


FIGURE 5.11c. Percent of flock walking under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

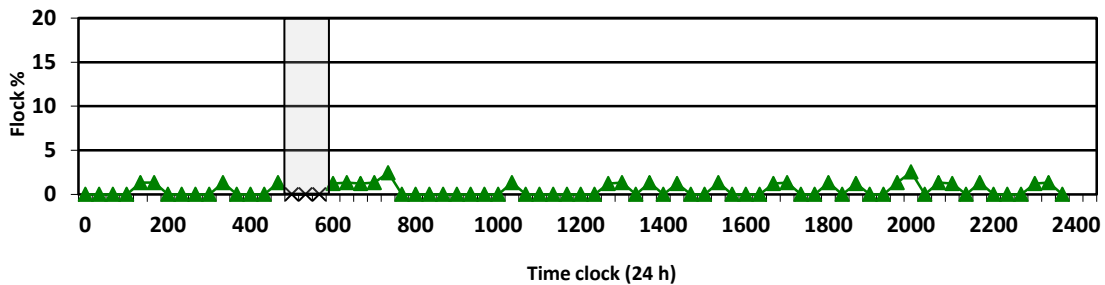


FIGURE 5.11d. Percent of flock walking under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

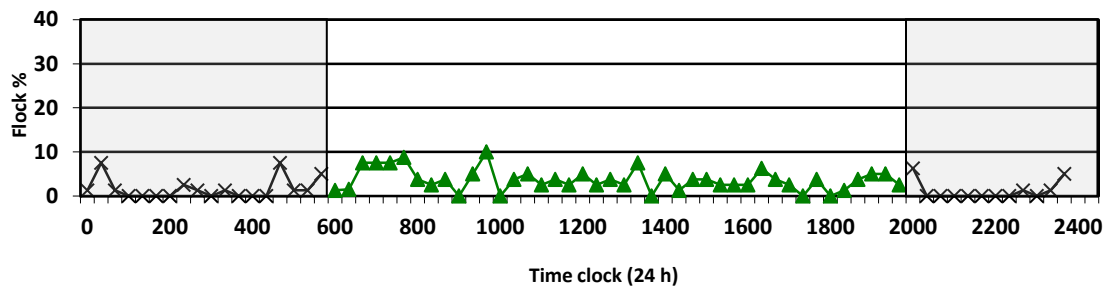


FIGURE 5.12a. Percent of flock standing under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0178$).

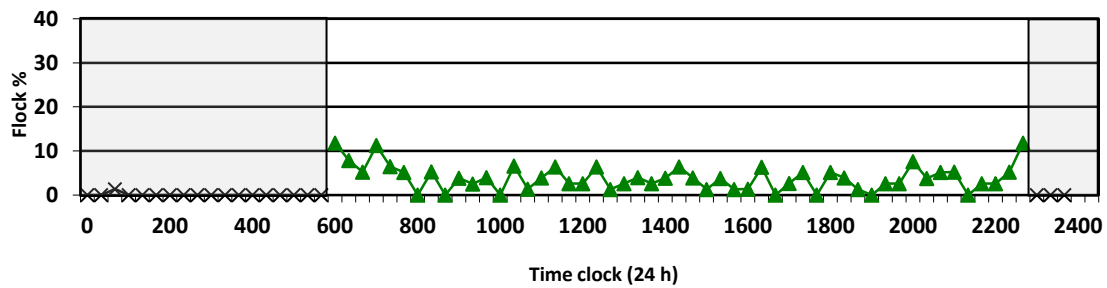


FIGURE 5.12b. Percent of flock standing under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0361$).

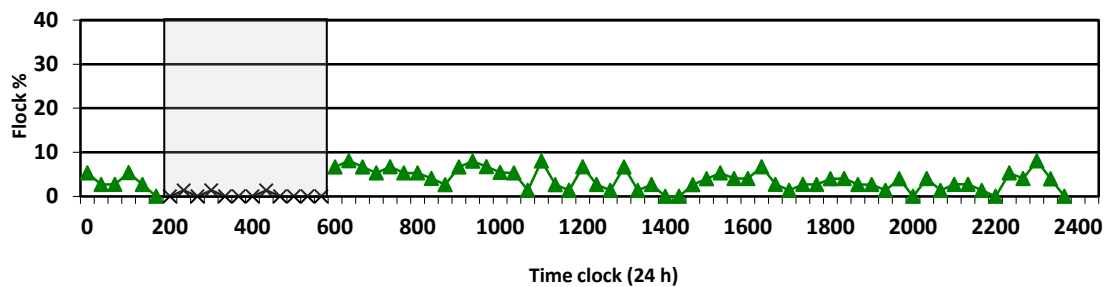


FIGURE 5.12c. Percent of flock standing under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds linearly with time during the photophase ($P=0.0001$).

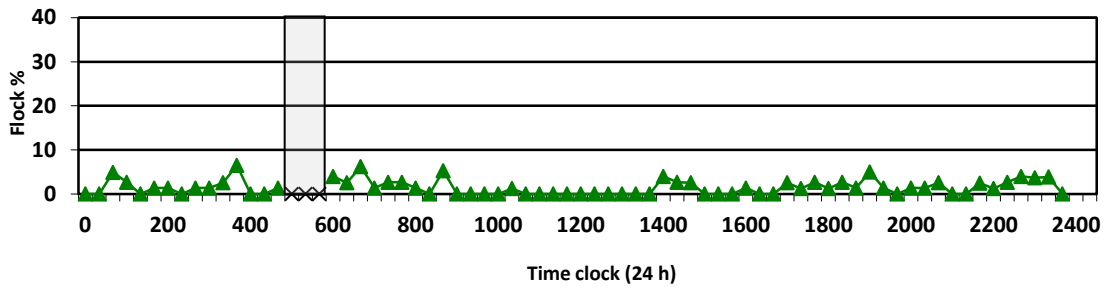


FIGURE 5.12d. Percent of flock standing under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

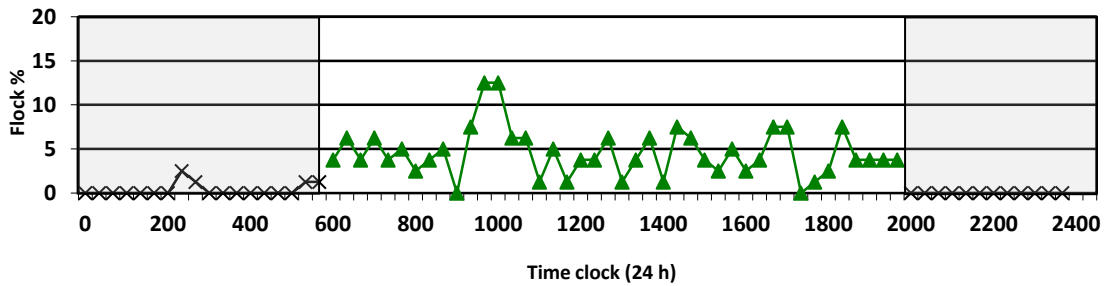


FIGURE 5.13a. Percent of flock preening under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

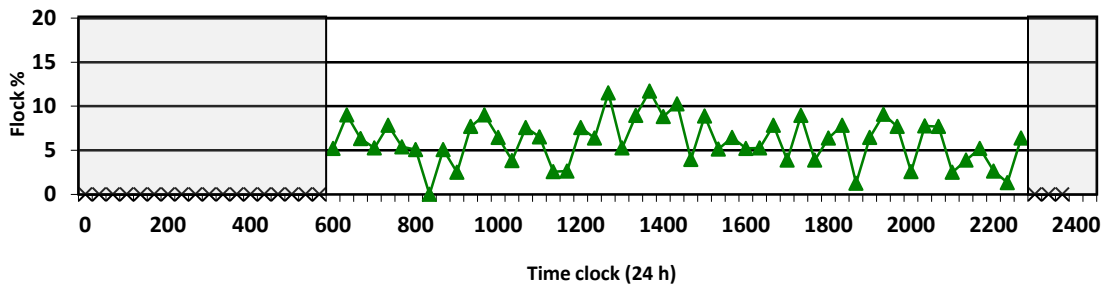


FIGURE 5.13b. Percent of flock preening under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

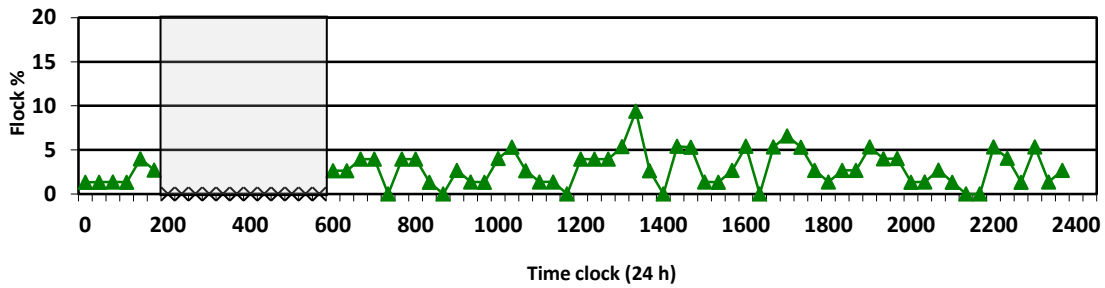


FIGURE 5.13c. Percent of flock preening under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

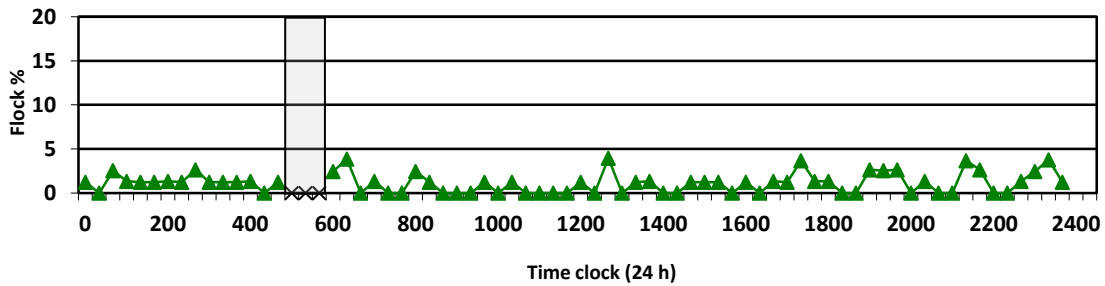


FIGURE 5.13d. Percent of flock preening under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

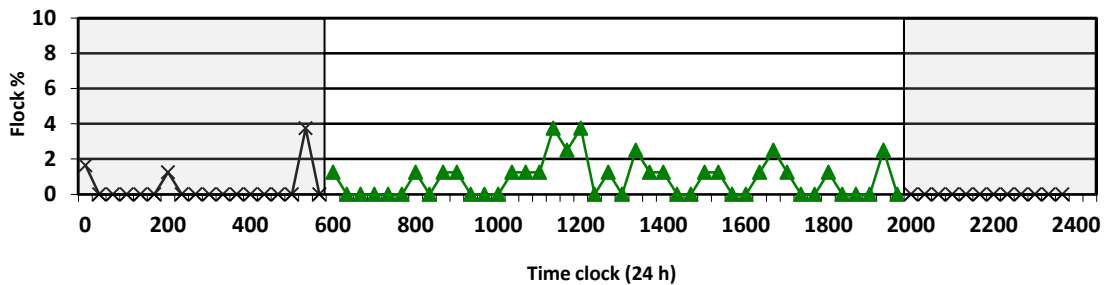


FIGURE 5.14a. Percent of flock stretching (wing and/or leg) under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0348$).

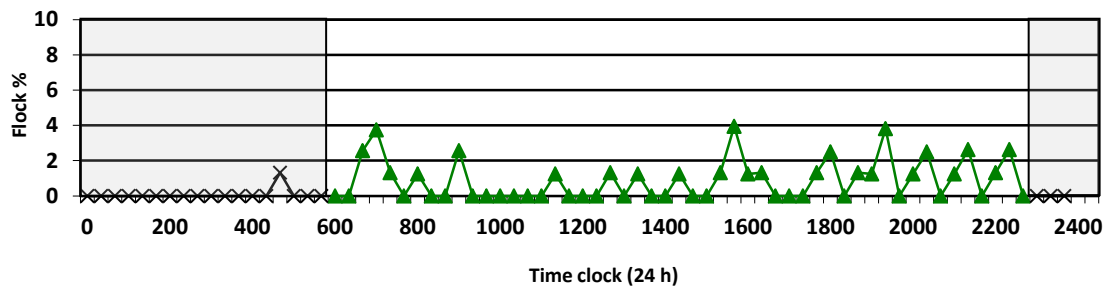


FIGURE 5.14b. Percent of flock stretching (wing and/or leg) under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

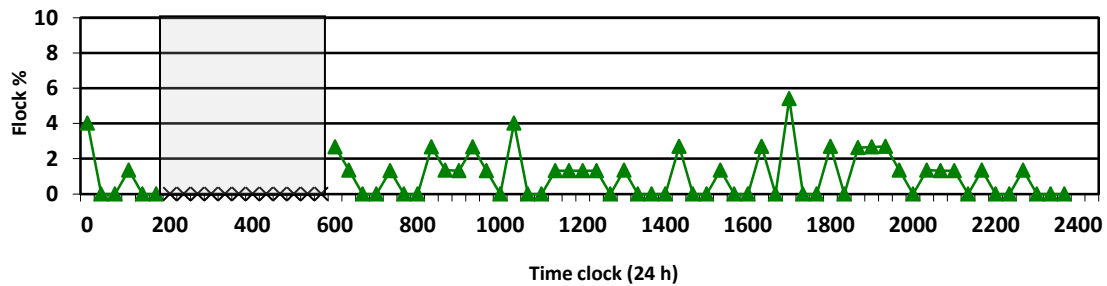


FIGURE 5.14c. Percent of flock stretching (wing and/or leg) under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

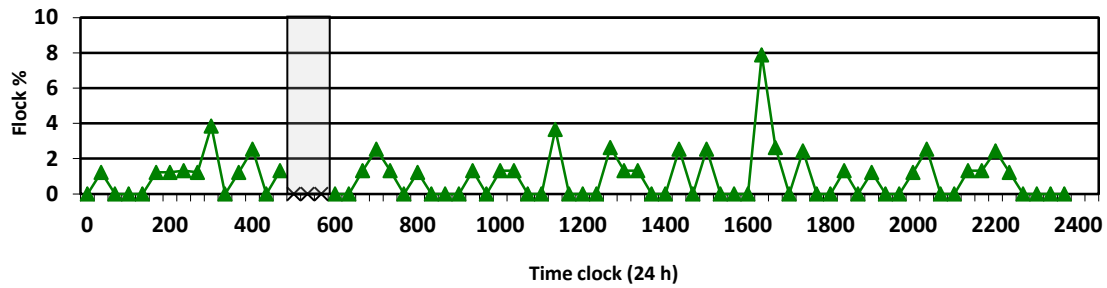


FIGURE 5.14d. Percent of flock stretching (wing and/or leg) under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

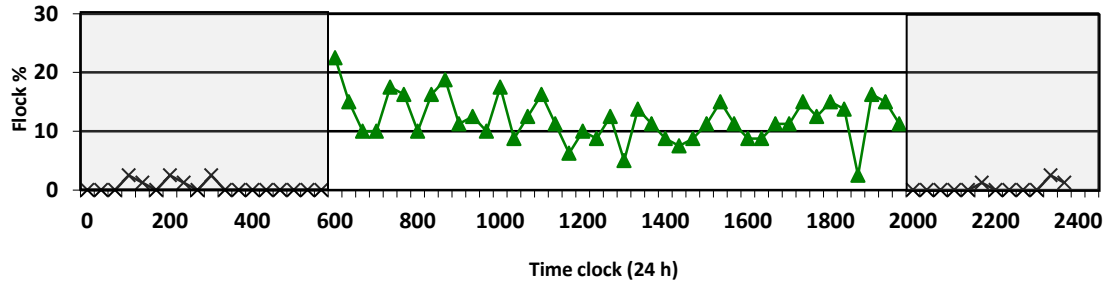


FIGURE 5.15a. Percent of flock at the feeder under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0361$).

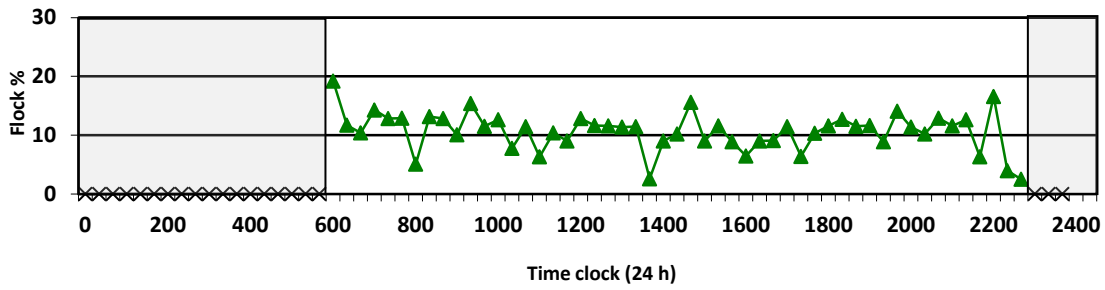


FIGURE 5.15b. Percent of flock at the feeder under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

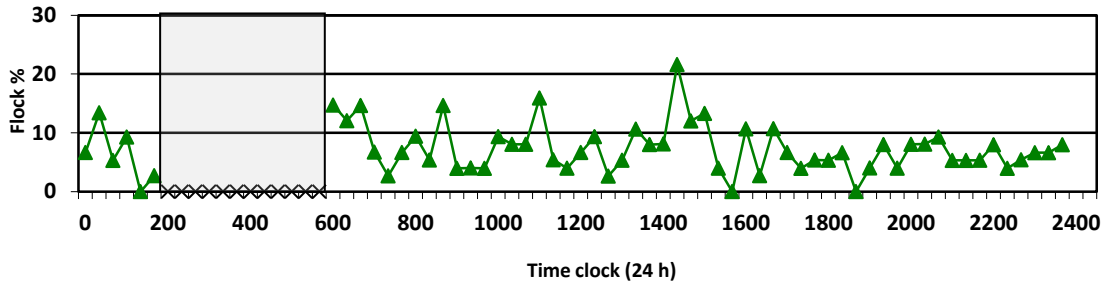


FIGURE 5.15c. Percent of flock at the feeder under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

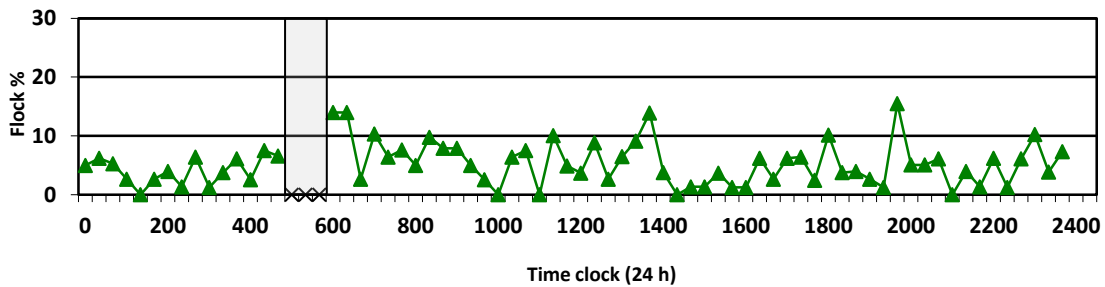


FIGURE 5.15d. Percent of flock at the feeder under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

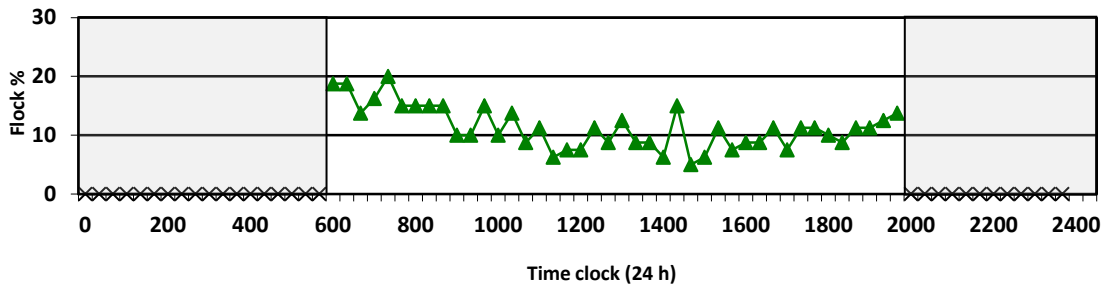


FIGURE 5.16a. Percent of flock at the drinker under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

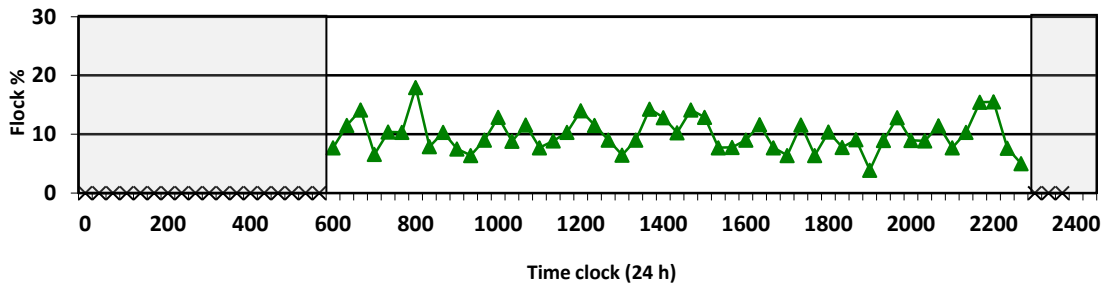


FIGURE 5.16b. Percent of flock at the drinker under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

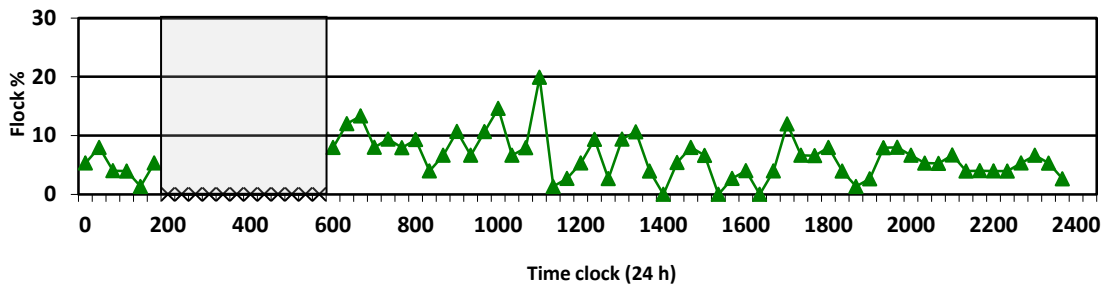


FIGURE 5.16c. Percent of flock at the drinker under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

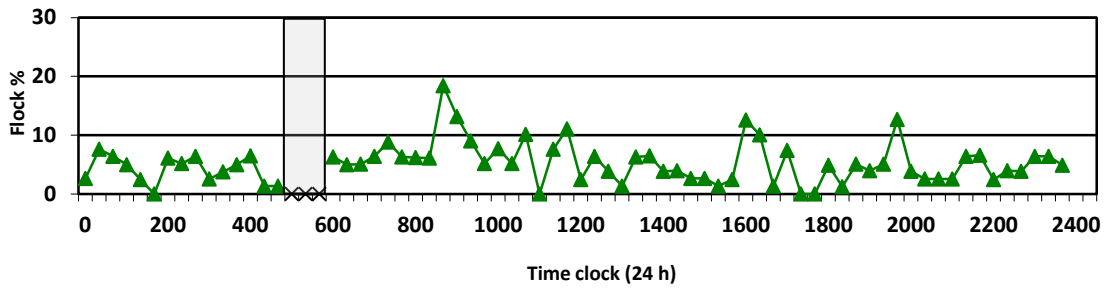


FIGURE 5.16d. Percent of flock at the drinker under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

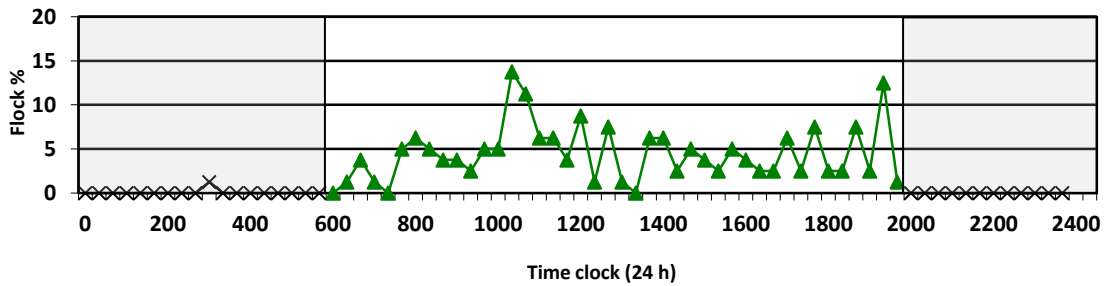


FIGURE 5.17a. Percent of flock litter pecking under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0333$).

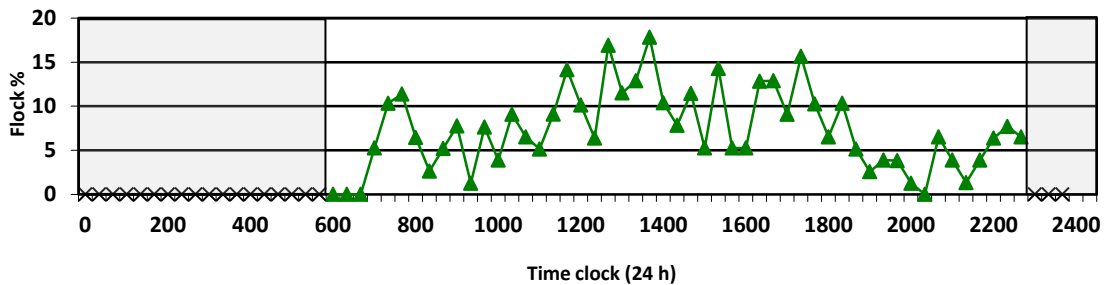


FIGURE 5.17b. Percent of flock litter pecking under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0001$).

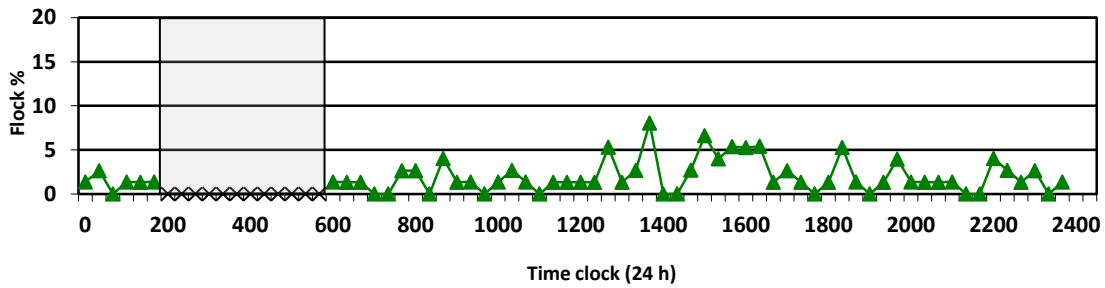


FIGURE 5.17c. Percent of flock litter pecking under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

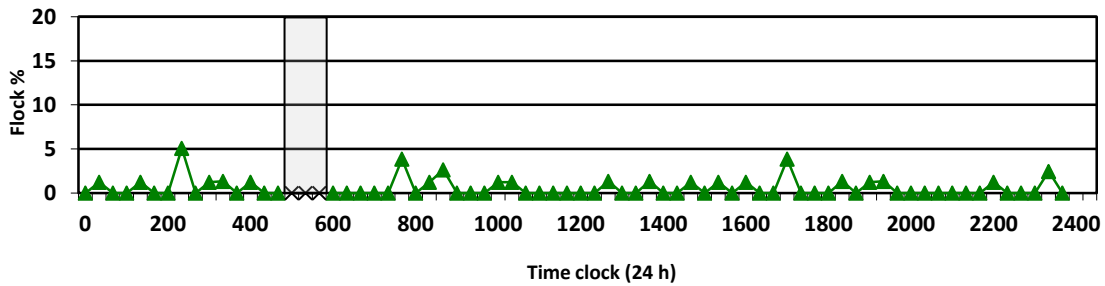


FIGURE 5.17d. Percent of flock litter pecking under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

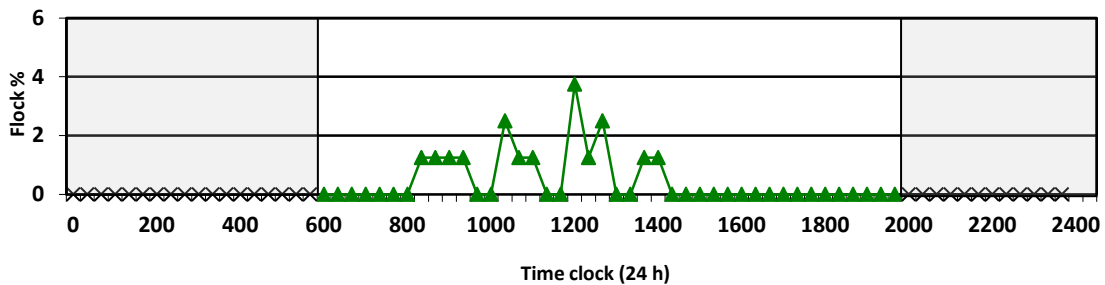


FIGURE 5.18a. Percent of flock dustbathing under 14L:10D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0335$).

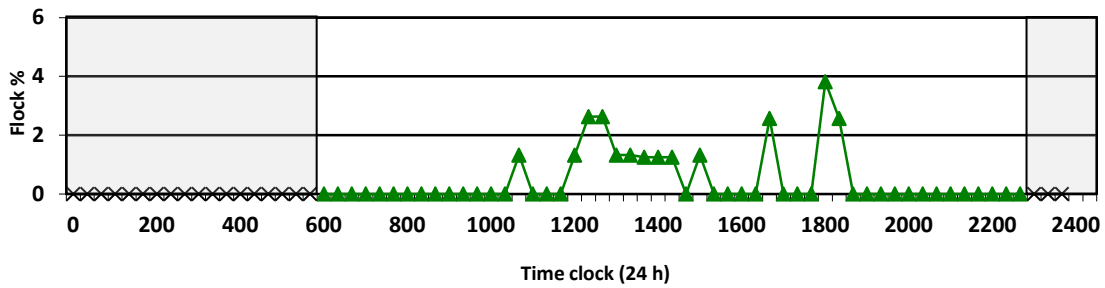


FIGURE 5.18b. Percent of flock dustbathing under 17L:7D at 42 d. The black line represents the scotophase period, and the green line the photophase. Behaviour responds quadratically with time during the photophase ($P=0.0004$).

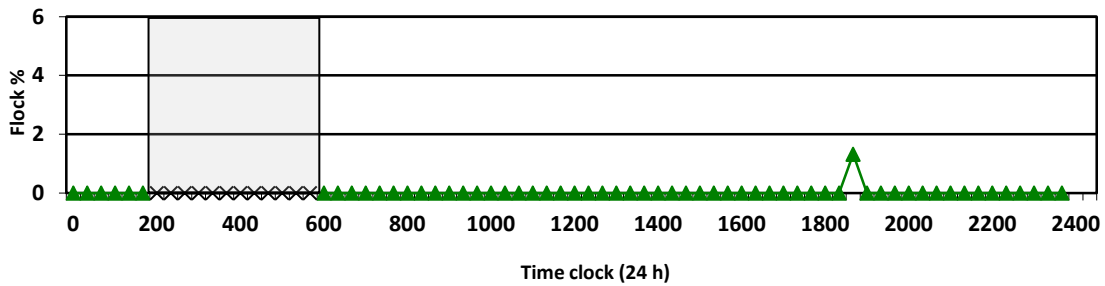


FIGURE 5.18c. Percent of flock dustbathing under 20L:4D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

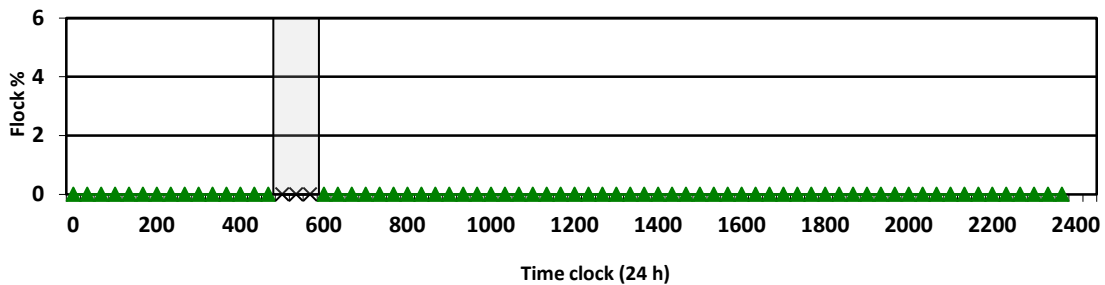


FIGURE 5.18d. Percent of flock dustbathing under 23L:1D at 42 d. The black line represents the scotophase period, and the green line the photophase. Daytime relationship between behaviour and time was not statistically significant.

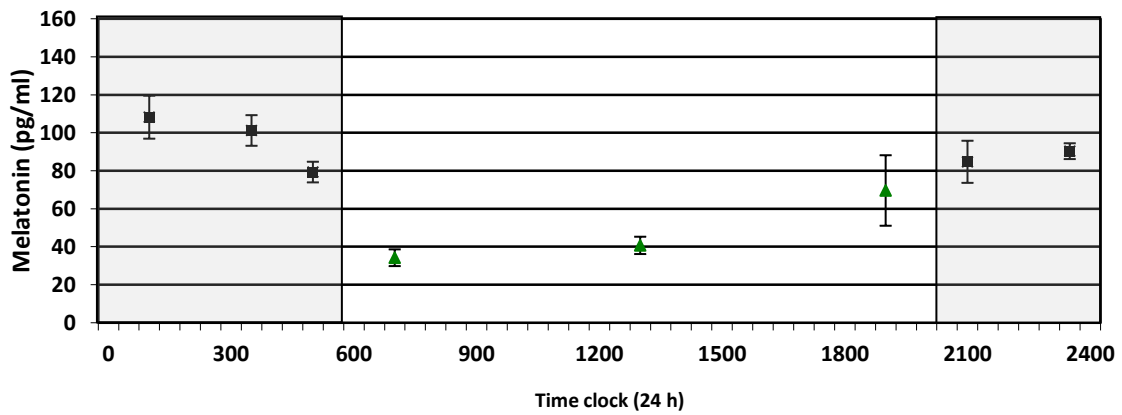


FIGURE 5.19a. Melatonin rhythm of birds raised on 14L:10D at d21. The black line represents the scotophase period, and the green line the photophase. Serum melatonin levels respond quadratically over the 24-h period.

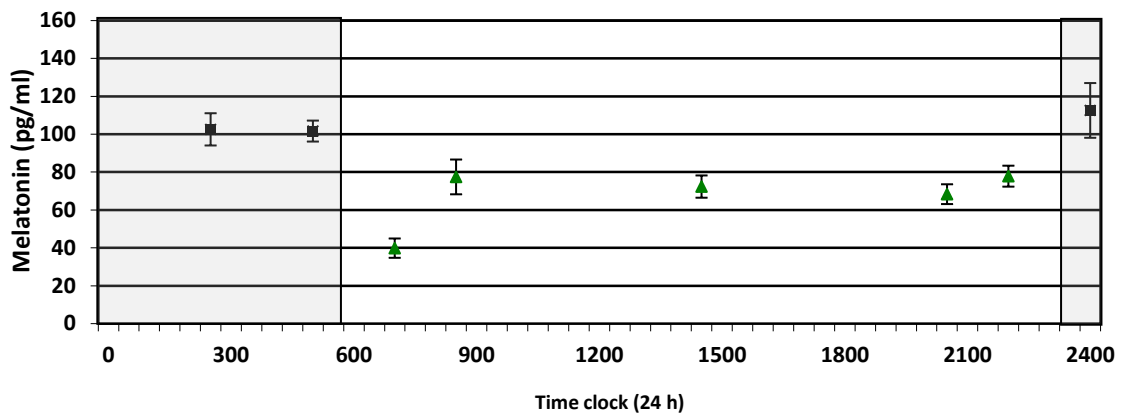


FIGURE 5.19b. Melatonin rhythm of birds raised on 17L:7D at d 21. Serum melatonin levels respond quadratically over the 24-h period.

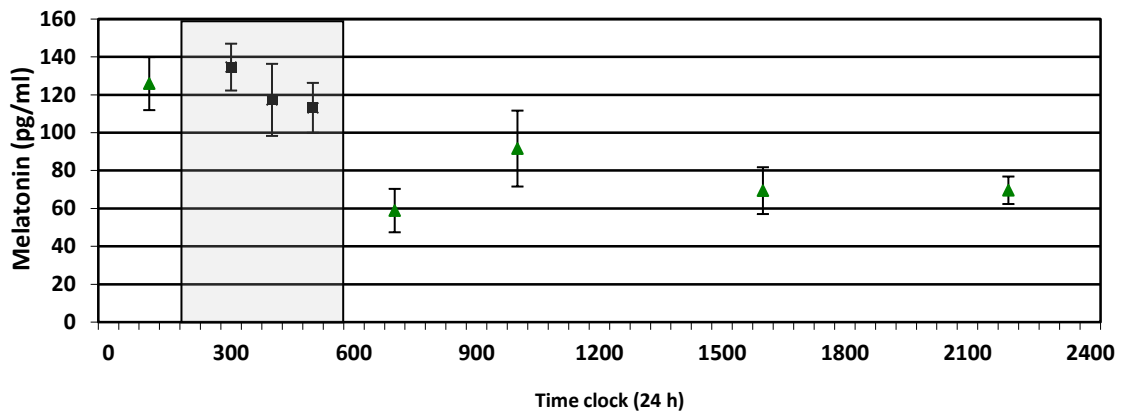


FIGURE 5.19c. Melatonin rhythm of birds raised on 20L:4D at d 21. Serum melatonin levels respond quadratically the 24-h period.

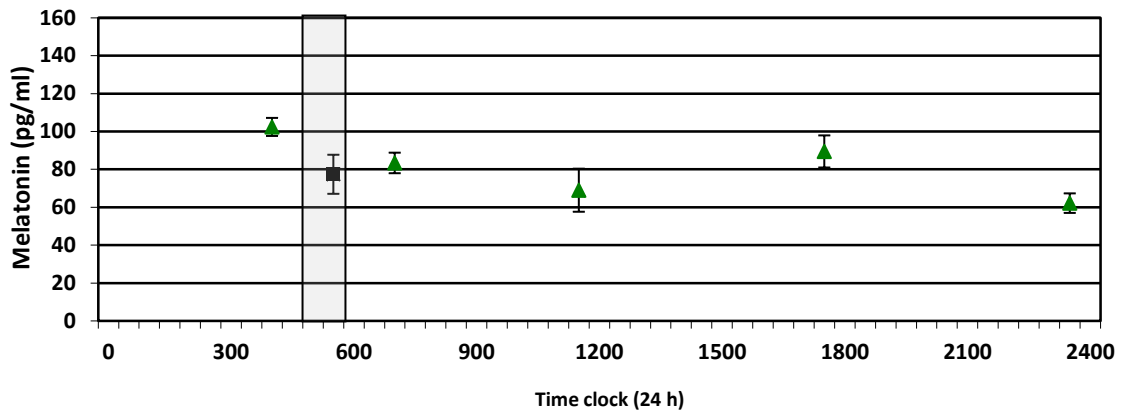


FIGURE 5.19d. Melatonin rhythm of birds raised on 23L:1D at d 21. The 24-h period relationship between behaviour and time was not statistically significant.

Chapter 6. Discussion

Introduction

There is a plethora of lighting programs used throughout the world, and in most cases, the decision to use a specific program has been based primarily on industry beliefs. The use of constant or near-constant light was traditionally chosen because of the visual access it allowed to birds. With birds seeing their environment throughout the day, it was believed that they would eat and drink for longer periods of time, and therefore be heavier by market age. This program was also chosen by many producers because birds were “quieter” when exposed to constant or near-constant light, making it physically easier to catch them at shipping. Welfare of the birds was likely not a consideration.

Lighting programs, when used, have generally included some darkness, but for the most part the length of darkness was not selected on the basis of a systematic scientific approach. The majority of the past research on lighting programs has compared one specific program to another, with little attempt to develop a comprehensive approach to understanding lighting effects. While this approach has helped develop a general insight of lighting program effects, it failed to develop a more thorough understanding of the relationship between the length of darkness, or its alternate daylength, and the welfare and productivity of broiler chickens.

To date, there are few legislated regulations on the nature of lighting programs to be used in the broiler industry. One example of a legislated regulation is found in the European Union (EU, 2007), where broilers must be exposed to not less than 6 h of darkness per day by at least 7 d of age. Within that 6 h, the darkness may be split into sections with one section containing at least 4 h of uninterrupted darkness. New Zealand is currently developing regulations for broiler welfare, which will be enforced by law. Of

significance is that New Zealand has already used the data from this work in the development of their regulations. In other jurisdictions, codes developed either by government or industry may be in place. In Canada, the Chicken Farmers of Canada (Chicken Farmers of Canada, 2009) have developed Animal Welfare Guidelines that are in the early stages of implementation. Requirements for exposure to darkness are not extensive, and broiler producers are only required to provide 1 h of darkness per day. To provide scientific evidence to serve as a basis for recommendations, a more systematic research approach is required, including on the effects of daylength.

Objectives

The objectives of this work were to study and understand how daylength impacts the welfare of commercial broiler lines, with the goal of establishing lighting programs that balance welfare and productivity. To do this, it was fundamentally important to use graded levels of daylength as experimental treatments that permit regression analysis to be used to determine the relationships between response criteria and daylength. The daylength treatments used were 14L:10D, 17L:7D, 20L:4D and 23L:1D. There are suggestions that daylength impacts genotypes differently, so two commercial genotypes were used in the majority of this work. It was also of interest to determine if males and females responded differently to daylength. Therefore, the primary experiments were designed with lighting program, genotype and gender as main effects. Lighting programs were replicated a minimum of 4 times to enhance the value of statistical analysis for productivity and mortality traits. A second experiment used whole room replications rather than small pens, so only 1 strain and mixed sexes were used. The age of marketing of birds varied, and included 32, 38 and 49 d of age. Having this variety within the experimental design permitted a better understanding of lighting effects and response prediction for broilers used to service various markets.

Measuring welfare is not simple, and the philosophy applied to this work is that the assessment should involve a wide variety of response criteria rather than focusing on only one or a few areas. Therefore, a number of response criteria were evaluated, which are centred on the 5 Freedoms that were recently reviewed and expanded (FAWK, 2011). They were:

1. Freedom from thirst, hunger and malnutrition by ready access to fresh water and a diet to maintain full health and vigour – To aid assessment, body weights were monitored in all trials, and health assessed as described below,
2. Freedom from discomfort by providing a suitable environment including shelter and a comfortable resting area – Assessment included factors which might arise from an inadequate environment and cause discomfort, such as mobility and foot pad lesions,
3. Freedom from pain, injury and disease by preventing or rapid diagnosis and treatment – Measurements included health parameters, including the incidence and cause of death loss, bird mobility and eye size,
4. Freedom to express normal behaviour – by providing sufficient space, proper facilities and company of an animal's own kind - Assessment included behavioural criteria, focusing on nutritive, exercise, comfort, and exploratory behaviours. Patterns of behaviour and melatonin rhythms were also examined as assessments of the development of circadian synchronization within a flock,
5. Freedom from fear and distress by ensuring conditions which avoid mental suffering – Tonic immobility was measured as a direct measurement of fear.

Positive behavioural expressions, such as comfort behaviour and play behaviour, were also monitored.

Since excellent productivity is a requirement of a successful producer, we have also measured other production criteria, including body weight, feed consumption, and feed efficiency.

Productivity

Productivity, including growth rate and feed intake, should rarely if ever be used as the sole criteria of animal welfare. However, in situations where animals are placed in an environment which theoretically should provide the ability for maximum growth, poor productivity can certainly signal that well-being is compromised. Birds raised under 23L:1D in this work were indicative of this issue. It is the provision of an environment in which birds have visual ability to see and be mobile for virtually the entire day (23 h light per day), which caused producers to believe that birds would grow at their fastest, yet this did not occur regardless of market age. Although it has been established that birds grow faster early in life on long photoperiods, even by 32 d, birds exposed to 4 h of darkness per day grew faster than the near-constant light birds. As the grow-out cycle was lengthened and birds marketed at older ages, birds clearly adapted to the dark period, and by 49 d, even those exposed to 10 h of darkness per day were as heavy as those in the more “visual” 23L environment. While these data clearly signal a problem with the birds exposed to near-constant light, it is not possible to identify one lighting program that is superior in growth rate for lighting programs using between 4 and 10 h of darkness. In fact, the data indicate that daylength selection for growth rate should be based on the age of broiler marketing. As the grow-out cycle is lengthened, birds are

able to adapt to longer periods of darkness, and with that comes the health benefits seen with use of shorter daylengths.

Mortality

More birds died when exposed to near-constant light, and the impact was reduced linearly regardless of the length of the grow-out period. Although statistics showed linearity in this response, it is interesting to note that numerically, mortality was similar or equal for birds under 14 and 17 L for the 7-32 d period, and actually higher for birds under 14L for the 7-38 d period as compared to those on 17L. These data indicate there is little to gain from a mortality perspective for using less than 17 h of daylength. The addition of 4 h of darkness to a lighting program produced intermediate mortality levels, and this shows that 4 h is not sufficient to provide for enhanced bird health.

Mortality levels generally are higher for faster growing birds. However, the shape of the mortality response curve did not follow the growth curve across all treatments, as mortality was highest for birds under 23L, yet these birds were never the heaviest. This helps clarify that body weight at age of marketing is not the only factor involved in influencing mortality levels. The increase in mortality associated with long daylength was primarily due to leg weakness and metabolic disease.

Mobility

Mobility in broilers is often scored using either the Kestin et al. (1992) or Garner et al. (2002) technique. Both scoring systems separate mobility into one of 6 categories, however definitions within the Garner et al. system are more precise, allowing better reliability between testers and between tests (Garner et al., 2002).

Previous research has shown that birds in the upper gait score categories of 3, 4 and 5 as measured on the scale established by Kestin et al. (1992) are birds in pain and the present data show that the percentage of birds falling into these categories are reduced by shorter daylength. The process of gait scoring is a subjective measurement, but in this work, the response to darkness in gait score is similar to the response seen in mortality due to leg weakness. This validates the gait scoring data. The impact of daylength on average gait score was either linear or, in the case of measurements at 38 d, quadratic, where the difference was small between birds on 14 and 17L. This is similar to the results seen for mortality and may signal that improvements level off between these daylengths, and that very long dark periods such as 10 h do not further benefit leg weakness.

Behaviour

The graded levels of daylength used in this work caused significant changes to the behaviour of the broilers. These changes are indicative of the welfare of the birds, and may have indirectly contributed to other welfare indicators such as bird mobility, and litter quality affected foot pad lesions.

Activity in general increased as daylength decreased. Statistics indicate a linear impact on inactive resting regardless of age tested, with birds raised under 23L being inactive for the highest percentage of time over the 24 h observation period. Despite the linear statistical interpretation, birds receiving 14L rested for a numerically longer percentage of time than did birds in the 17L treatment. This suggests that a quadratic response to daylength may have occurred if more daylengths had been included in the experimental design, particularly shorter daylengths. Because birds are for the most part inactive during the night period, a quadratic response is likely expected as a larger

portion of the 24-h period is taken up by darkness with shorter daylengths. Extreme lethargy has been suggested to be a negative welfare indicator in birds, and while it is difficult if not impossible to say what level of inactivity is actually detrimental (or what level of activity is maximizing welfare for that matter) it could be expected that birds provided with the visual access to their environment for the majority of the day would make use of that environment. Hence, it is highly probable that the level of inactivity noted with 23L birds signifies a welfare issue.

Since the birds receiving 23 h of light per day are the most inactive, obviously they spent less time performing all other activities. Walking and standing are among these activities and the reduced expression of these behaviours may have affected other aspects of bird welfare. For instance, there is evidence in a number of species that increased exercise improves skeletal quality. The increase in mortality and culling due to leg weakness is supportive of a connection, as is the reduction in mobility seen for the 23L birds. However, establishing a cause-effect relationship is not possible with these data. Did birds on shorter daylengths exercise more because their skeletal systems were stronger, or did the exercise make their skeletal systems stronger? When considering the reason for the reduced standing and walking in 23L birds, another possible explanation is that they exercised less because they were less mentally aware of their environment. This will be discussed further in relationship to sleep and sleep deprivation. Reduced standing and walking can also relate to feeding behaviour, and longer daylengths were associated with a less amount of time spent at the feeder. It is interesting to remember that the traditional reason for using near-constant light was to provide a visual environment so that birds could maximize time at the feeder.

A reduction of behaviours that are highly motivated in an organism is considered to be indicative of reduced welfare. In all broilers, eating is a highly motivated event (Bokkers et al., 2004), although it appears that the rate of growth may impact the motivational factor. However, Bokkers and Koene (2004) found a relationship between speed of growth and motivational expression of walking to a feeder. They stated that slow growing broilers were controlled by motivation, but fast growing birds, although motivated to feed, were more controlled by a physical inability, which controlled walking to a feeder.

The data from this work show a slightly different trend. Birds given longer periods of darkness did spend more time at the feeder, but did not consume more feed than birds given near-constant light. In addition, the birds exposed to 23L:1D were never the heaviest, and at older ages, weights were similar to broilers exposed to 14L:10D. This signals that increasing daylength results in a change to the behavioural pattern of eating. The reason for the reduction in percentage of feeding time can't be precisely defined, but could relate to physical ability (as shown in this research) and/or motivation to eat due to sleep deprivation. Regardless of mechanism, the reduction seen in percentage of feeding time is likely a strong indicator of reduced welfare for a variety of reasons.

Drinking behaviour follows a different trend as compared to feeding. At 28 d of age, there is no impact of lighting program on time present at the drinker over the 24-h period of time, suggesting that drinking is a vital behaviour that birds are highly motivated to perform. As birds get older, time at the drinker is reduced, which is in agreement with reduced expression of some behaviours as birds age (Bokkers and Koene, 2004). The impact of daylength on drinking behaviour also changes with levels

of the behaviour decreasing as the amount of darkness exposure decreases. Reasons for the change in response may relate to the reduced mobility of older birds, and therefore greater difficulty in getting to the drinker. For those birds in gait score categories greater than 2, pain may reduce the motivation to move and as noted above, the incidence of birds in these categories increases with increasing daylength. However, this is a relatively low percentage, hence does not explain all of the variation. Other factors such as bird weight and housing density, which both increase with bird age, can affect behaviour but they do not explain daylength differences as the response trends for these characteristics are not affected in the same way as drinking behaviour. Lack of motivation due to the proposed sleep deprivation for birds given long daylengths is a strong possibility as an explanation for reduced drinking. No drinking occurred at either age under any lighting periods during the scotoperiod.

The percentage of time spent in comfort and exploratory behaviours also signifies a change in the well-being of birds exposed to the various photoperiods. Peak percentages of time were linear or quadratic for preening, dustbathing, stretching and litter foraging regardless of the age measured. The incidence of these behaviours performed by birds under 23L was either non-existent or at a very low incidence, signifying poor welfare as compared to birds on other photoperiod programs. Once again, those birds given 20L were only intermediate to those on 14 or 17L. There is no clear separation in the maximum percentage of these behaviours performed by birds on either 14 or 17L.

Running is sometimes considered a play related behaviour for poultry, and play behaviours, like comfort behaviours, are performed when other basic needs of the animal are met. The use of near-constant light has virtually eliminated the behaviour,

and once again, as body weight is not highest for this group, fast growth cannot be the only factor involved. Although the reduction or elimination of this behaviour on its own cannot signify poor welfare, it is a possible indicator.

An increase in activity may have indirect impacts on some aspects of bird health. For example, higher activity levels may result in better litter quality, which in turn can mean a lower incidence or severity of foot pad lesions. However, while activity differed between 20 and 23L, average lesion scores were actually numerically higher under 20L at both 35 and 45. It is possible that body weight is a confounding factor here, but may also suggest that activity levels need to be considerably higher in order to extrapolate differences into better litter quality. Similarly, an increase in exercise has been suggested to reduce the incidence of leg weakness in broilers. While there is likely some association between the two, it is hard to understand if the increase in exercise is a result of better leg health due to some other factor, or if the exercise causes an improvement in leg health. A comparison between the behavioural data and the gait score data show that the relationship is certainly not exact. At 28 d, activity levels are quite different between 17 and 20L for example, but the percentage of birds falling into the painful category of 3+4+5 is identical. At 45 d, the numerical percentage of birds in these categories is actually higher under 17L.

Behavioural results in this thesis provide evidence on the length of darkness exposure required to meet the biological needs of broilers. Providing 10 h of darkness to broilers changes behaviour, and in some cases, these birds spend a lower percentage of their time in active behavioural expression than do those birds exposed to 7 h of darkness. It is possible that birds simply did not have enough daylight hours to perform all of the behaviours in their repertoire during the short 14 h days. This difference is not

as obvious at 42 d as compared to 38 d. Overall behavioural activity expression at the older age is reduced; therefore more allowable time for the birds under 14L could explain why activity was higher for these birds at the older age. Evidence can also be found by examining darkness behaviour in this treatment. Although behavioural expression is low, both feeding and stretching occurring during the scotoperiod at both ages under 14L. The data indicate that birds are hungry before the long 10 h dark period ends, or it is possible that enough sleep is achieved before the period is up, and therefore birds are starting to wake and become more active. Both of these observations suggest that the biological needs for darkness exposure and presumably sleep is met at approximately 7 h duration.

The simple test of examining the percentage of birds moving away from the observer indicates response that is similar to behavioural testing – that birds on 23L are less reactive than those on all other photoperiods, and those on 20L are only intermediate between shorter daylengths (14L, 17L) and 23L. This could be reflective of less fear in the birds exposed to longer daylength. The tonic immobility testing did not show a significant difference in fear levels between birds on any of the four lighting programs, but variability in this test was quite high. Numerically, birds on the 23L stayed in a tonic state for a shorter period of time, which had it been significant, would indicate a reduced fear (Jones and Faure, 1981). However, alternate explanations for this behavioural response are equally possible or more likely. Sleep deprivation, which will be discussed later, results in a reduction in reactivity in many species. In fact in humans, sleep deprivation can result in poorer reactivity than when individuals have a blood alcohol level of 0.057 (Powell et al., 1999). If that is the case, then these responses are related to birds not recognizing a generally fearful situation. The sound data may support this – birds under near-constant light are much quieter than birds allowed

darkness exposure, indicating a reduction in communication between the flock-mates.

While the sound data alone is not conclusive of a reduction in welfare, it is supportive of that conclusion. Level of sound in flocks with a dark period of 4 h or more is similar, but near-constant light results in a flock that is much quieter.

Behavioural and physiological rhythms

Both the behavioural and melatonin production data show that the hours of daylength impact circadian rhythms within a flock. Quadratic relationships in melatonin production were found under 14, 17 and 20L, although the significance of the regression weakened as daylength increased. There was no relationship found under flocks raised under 23L. Similarly, behavioural patterns followed clear day-time rhythms under 14L and 17L, only occasionally under 20L, and never for 23L birds. These data strengthen the pattern seen that 20L is intermediate in its response, and may not provide enough dark time to maximize welfare of broilers.

How changes to melatonin production impact physiological changes is not well understood, but hypothetical mechanisms include level of peak production, duration of elevated levels, and sensitivity of particular organs (Reiter, 1987). Prior research has shown that lengthening the dark period increases the duration of elevated melatonin production in turkeys (Zawilska et al., 2007), and response curves in this work follow this trend. Growth of eyes occurs during the day period, so the duration of high melatonin production likely has impacted weights in this work. Birds exposed to 14L, 17L or 20L had eye weights similar to each other, but those exposed to near-constant light had significantly heavier eyes. It cannot be stated that the heavier eye weights found in birds raised under 23L in this work were a welfare concern, as pain and other symptoms of

eye disease were not assessed. In other species exposed to constant light, eye size is abnormally large (Barnett et al., 1971; Davis et al., 1986). Over a long period of time, this can result in pressure on the optic nerve, causing discomfort or even pain, and the resulting change in eye shape can result in glaucoma. However, in birds marketed at young ages, this may not be the case. Assessing the pain implications of increased eye size is beyond the scope of this study, but large eye size is an abnormal situation, and further strengthens the argument that near-constant light is not appropriate for broiler production. Unlike other assessments of bird welfare, eye size does not differentiate between 14, 17 and 20L treatments.

Sleep

This study did not follow circadian patterns within a single bird, but evidence from a number of species indicates these rhythms are free-running and not synchronized in individuals exposed to constant light. As a consequence, synchronization of behaviour and physiological rhythms within a group of animals would also be lacking. This is important for a number of behaviours, including sleep. If broilers within a flock follow their own free-running rhythm, then birds sleep at various times during the photo- and scotophases. While this in itself may not be a problem, it is very possible that birds attempting to sleep are repeatedly being awoken by those on a different rhythm that are eating, drinking, or performing other behaviours.

Repeated awakenings can result in a type of sleep deprivation termed sleep fragmentation and the lack of behavioural rhythms in 23L broilers indicates that this was occurring in the present research. The lack of a circadian rhythm in melatonin, the hormone known to promote sleep, and the reduced quality of sleep that occurs during the photophase (Rattenborg et al., 2005) further support a sleep deprivation hypothesis.

In turn, sleep deprivation could be an explanation for many of the negative effects seen for broilers exposed to long daylengths.

Chronic sleep fragmentation can lead to symptoms that are similar to those for total sleep deprivation (Bonnet, 1986; Chen and Kushida, 2005) and these symptoms are variable. Carbohydrate metabolism is affected, with the speed of blood glucose clearance being reduced when adequate sleep is not achieved (Speigel et al., 1999; Copinschi, 2005). This can affect the efficiency of feed utilization and may be a portion of the reason for reduced feed efficiency seen for broilers given long daylengths in the present study.

Sleep deprivation results in negative impacts on the health of an animal, and in rats has been shown to lead to muscular weakness, an increase in the incidence of footpad lesions, pathological disease, higher mortality levels, (Rechtschaffen et al., 1983), and an increased stress response (Everson et al., 2008). In the present work, footpad lesions, metabolic and leg weakness disease and mortality levels were higher in birds exposed to longer day periods. Behaviourally, sleep deprivation reduces the alertness of an animal (Powell et al., 1999; Powell et al., 2001; Boerema et al., 2003), which was suggested in this work in the lack of responsiveness of the 23L birds and to lesser extent broilers receiving the 20L treatment. Reduced alertness has been linked to less effective synaptic activity in the brain (Chen and Kushida, 2005). A lack of responsiveness through poor brain functioning would explain why birds under those photoperiod programs did not respond to an observer to the same degree as the birds allowed a longer dark period. A general lack of alertness would also decrease activity of an organism, and this was seen as increased resting for birds given long daylengths with a concomitant decrease in most behavioural expression.

Practical applications of lighting programs

This study examined the use of daylength for broiler production ranging from 14 h per day to 23 h per day, using graded levels to establish response curves in a wide range of welfare and productivity parameters. The primary objective of this work was to determine the impact of graded daylengths on the welfare of broilers, but it was also of interest to be able to recommend a lighting program for commercial broilers that balances welfare and productivity of broilers over a range of marketing ages and environmental conditions. The market ages tested were between 32 and 49 d. The results indicate that one specific daylength cannot be recommended for all situations based on welfare and productivity for broilers. However, the data has given vitally important information on how daylength and exposure to darkness should not be used, and how birds respond to varying daylength. Therefore, these data serve as a strong base for the selection of daylength from welfare acceptable and production optimization standpoints.

Table 1 lists the response of welfare criteria measured in this work as impacted by daylength. The table includes data that is proportionately comparative to the response of the 23L:1D birds – hence the latter birds are expressed as 100% and other treatments are compared to that level. From this information, each criterion is then ranked according to bird welfare using a range of 1 to 4 with the higher the ranking, the poorer the assessed welfare.

It is clear that long photoperiods result in poor welfare. The 23L treatment ranked the poorest in health, largest eye size, mobility, behavioural expression, and response to observer criteria. A number of response criteria were not included in this table, as they did not fit the model used. Growth for example, was lower under shorter

days, but this is understandable. What does suggest a welfare reduction is the poorer growth noted in the visually optimal environment. Dustbathing activity at 42 d was not included, as flocks under 23L no longer dustbathed. Rankings on the other three programs indicated highest dustbathing activity under 17L, followed by 14L and 20L (nearing 0) respectively. Flocks under 23L treatment did not have a synchronized melatonin rhythm in the flock, and behavioural patterns also were not synchronized through the day, suggesting sleep deprivation and further documenting the poorer welfare of birds given this treatment. Increasing darkness to 4 h improved welfare parameters, but only marginally, as all response criteria ranked only slightly better than those found for 23L birds. Bird welfare improved with shorter daylengths but the differentiation between the 14L and 17L treatments was not always clear. Ranking for health parameters were better under 14L than 17L with respect to mortality, mobility, and footpad lesions. However, behavioural expression tended to be stronger under 17L than 14L. The response differences between these treatments were also proportionally small indicating welfare differences for daylengths in this range are not large, and this is supported by the statistical finding of a quadratic response for many criteria examined. These treatments also showed distinct behavioural patterns throughout the day and melatonin synchrony on a flock basis occurred under both photoperiod programs.

On a productivity scale, the choice of long photoperiod programs may also not be appropriate because of reduced growth and feed efficiency, and increased mortality. Figure 1 depicts growth rates over various ages, and shows that, although historically believed that 23L would provide maximum growth rate, this did not occur. Small lengths of darkness exposure resulted in higher body weights at young ages, and as birds adjusted to darkness exposure, even those with longer darkness exposure periods outperformed those birds raised on 23L. Not only does this depict better production levels

with darkness exposure, but it also signals a welfare issue when birds are raised under visually available areas with a corresponding reduced growth rate. Figure 2 shows gain to feed ratio, and again, this indicates that darkness exposure, particularly long periods, improves feed efficiency. Mortality, which was discussed above in relationship to welfare, also is a productivity and economic concern. Although not shown in this data set, condemnations and downgrading at the processing plant may be higher for birds given long daylengths (unpublished data). The only known economic advantage to using near-constant or constant light is found in muscle proportioning. In a concurrent study, breast meat yield was found to respond positively to increasing daylength (Schwean-Lardner et al., 2006c) and under specific market conditions this would be an economic factor that would be considered along with the production parameters noted above in selection of a daylength.

When evaluating the overall welfare status of broilers based on this research, approximately 17 h appears to be the maximum amount that should be included in a broiler production system. This is not an exact value because of differing responses in assessment criteria to the graded daylengths used in this research, but does give the majority of welfare benefits with minimal reduction in production parameters.

Particular circumstances in individual farms may aid in the decision factor for what daylength to include in a lighting program. For example, if mortality in a particular flock has historically been high, then including more darkness would be advantageous for both welfare and productivity, particularly if mortality has been due to metabolic or leg weakness issues. Areas of broiler production that are in high altitude areas typically have higher metabolic disease, and the inclusion of darkness for longer periods of time may be more important. Cost of feed is also important. As darkness can improve feed

efficiency, including more darkness can reduce costs. Age at marketing will also impact lighting choice as response to daylength for some criteria changes with age. For example, younger birds may not have time to adjust to darkness exposure, so the longer birds are kept, the more darkness could be added.

Finally, lighting recommendations for various genotypes differ from the respective Primary Breeders. However, in this work, regardless of the genotype or the gender, the inclusion of increasing levels of darkness impacted genotypes and genders similarly, pointing to a basic biological response in all birds measured.

Conclusions

In conclusion, welfare of broilers must be considered when choosing a daylength for inclusion in a photoperiod program. A near-constant photoperiod (23L:1D) has many negative impacts on the welfare of the broilers, and would not be recommended for these reasons. Since a four h darkness inclusion is only intermediate in its welfare improvements, longer dark periods should be used, although moving to 10 h, as used in this work, may not be required. Approximately 7 h (ranging on either side of this scale) would be a maximum amount recommended for most flocks, although specific flock conditions should be considered.

The data included in this thesis are being used by Aviagen to provide a base for a computer model that will be available to help in lighting program determination. The program will allow the input of production system parameter history, as well as income and expenses, to aid in the determination of an appropriate lighting program. Because so much of these data are of a welfare focus, the modelling system will also allow decisions to be based on welfare parameters. The program is well on its way to completion, and is meant to be used world-wide for broiler production systems.

TABLE 6.1. Proportional comparison (%) of response criteria based on values for birds on 23L:1D and ranking of bird welfare based on these values using a numerical range from 1 to 4, with 1 representing the best welfare and 4 the poorest .

	Daylength (h)							
	14		17		20		23	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
<u>Mortality</u>								
<u>Experiment 1</u>								
7-32 d	65.8	1	66.7	2	87.6	3	100	4
7-39 d	60.9	2	59.0	1	89.0	3	100	4
7-49 d	55.4	1	59.1	2	108.6	3	100	4
<u>Experiment 2</u>								
7-31 d	79.7	1	107.3	4	81.5	2	100	3
7-38 d	68.5	1	97.2	3	79.3	2	100	4
Mean mortality ranking		1.2		2.4		2.6		3.8
<u>Mean gait score (GS)</u>								
<u>Experiment 1</u>								
28 d	53.4	1	69.8	2	80.2	3	100	4
35 d	40.0	1	47.1	2	57.1	3	100	4
45 d	64.9	1	76.6	2	90.3	3	100	4
<u>Experiment 2</u>								
36 d	70.0	1	82.2	3	81.1	2	100	4
Mean GS ranking		1.0		2.3		2.8		4.0
<u>Foot pad lesion score (FPLS)</u>								
28 d	58.3	1	75.0	2	89.6	3	100	4

TABLE 6.1. Proportional comparison (%) of response criteria based on values for birds on 23L:1D and ranking of bird welfare based on these values using a numerical range from 1 to 4, with 1 representing the best welfare and 4 the poorest .

	Daylength (h)							
	14		17		20		23	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
35 d	85.7	1	89.3	2	107.1	4	100	3
45 d	94.1	1	102.0	3	109.8	4	100	2
Mean FPLS ranking		1.0		2.3		3.7		3.0

Eye weight

31 d	86.27	3	83.05	1	85.62	2	100	4
46 d	89.05	2	90.00	3	88.5	1	100	4

Behaviour (24 h)

Inactivity 27 d	90.6	2	85.5	1	98.8	3	100	4
Inactivity 42 d	85.8	2	80.5	1	91.8	3	100	4
Standing 27 d	159	2	207	1	130	3	100	4
Standing 42 d	207	2	195	3	235	1	100	4
Walking 27 d	190	2	258	1	132	3	100	4
Walking 42 d	420	1	528	2	312	3	100	4
At feeder 27 d	113	3	125	1	115	2	100	4
At feeder 42 d	144.1	2	152	1	120	3	100	4
Drinker 27 d	94.9	2	111	1	86	3	100	4
Drinker 42 d	132.4	2	145	1	110	3	100	4
Preening 27 d	224	1	206	2	140	3	100	4

TABLE 6.1. Proportional comparison (%) of response criteria based on values for birds on 23L:1D and ranking of bird welfare based on these values using a numerical range from 1 to 4, with 1 representing the best welfare and 4 the poorest .

	Daylength (h)							
	14		17		20		23	
	Value	Rank	Value	Rank	Value	Rank	Value	Rank
Preening 42 d	240	1	398	2	220	3	100	4
Stretching 27 d	1950	2	5500	1	550	3	100	4
Stretching 42 d	308	2	346	1	215	3	100	4
Dustbathing 27 d	273	2	387	1	107	3	100	4
Foraging 27 d	634	2	879	1	458	3	100	4
Foraging 42 d	481	2	975	1	324	3	100	4
Mean behaviour ranking		1.9		1.3		2.8		4.0
Response to observer – 30 d	510	3	646	2	654	1	100	4
Response to observer – 47 d	856	1	641	2	490	3	100	4
Sound	116	3	120	2	132	1	100	4
AVERAGE RANKING		1.6		1.8		2.8		3.9
RANKING		1		2		3		4

FIGURE 6.1. Comparison of body weight as compared to those from birds raised on 23L:1D (100%).

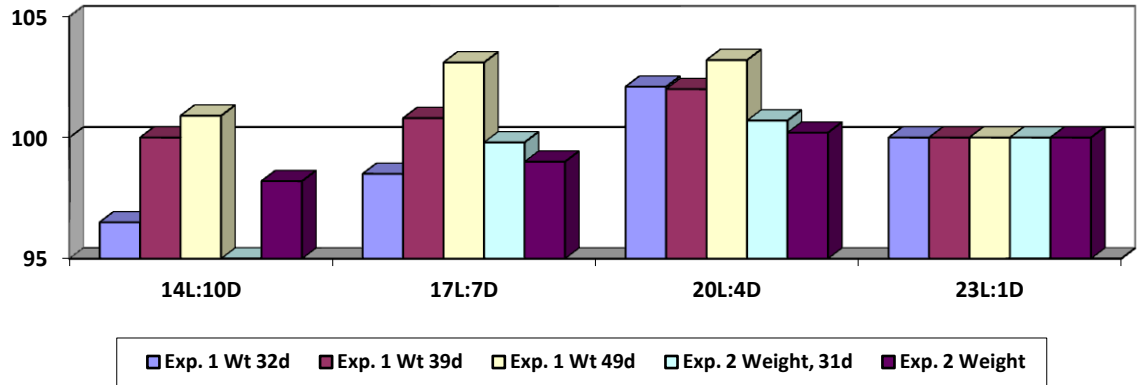
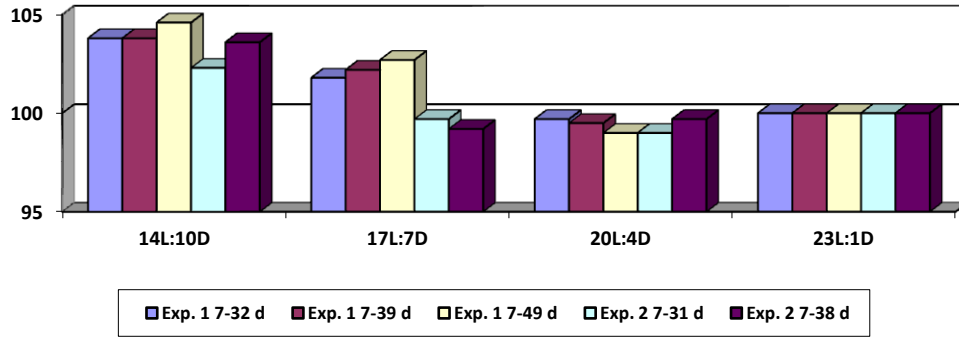


FIGURE 6.2. Comparison of gain to feed ratio as compared to those from birds raised on 23L:1D (100%).



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Chapter 8. Presentations

8.1 Peer reviewed publications

- K. Schwean-Lardner**, B.I. Fancher, and H.L. Classen. 2011. Impact of darkness exposure on productivity of two commercial broiler strains. *Br. Poult. Sci.* (in press).
- K. Schwean-Lardner**, B.I. Fancher, and H.L. Classen. 2011. Effect of daylength on behavioural and melatonin rhythms in broilers. *Appl. Anim. Behav. Sci.* (under review).
- K. Schwean-Lardner**, B.I. Fancher, B. Laarveld and H.L. Classen. 2011. Effect of daylength on flock behavioural patterns and melatonin rhythms in broilers. *Anim. Welf.* (under review).
- K. Schwean-Lardner**, B.I. Fancher, S. Gomis, A. Van Kessel, S. Dalal and H.L. Classen. 2011. Effect of daylength on cause of mortality, leg health, and ocular weight in broilers. *Poult. Sci.* (under review).

8.2 Conference presentations

- K Schwean-Lardner**¹, B.I. Fancher², and H.L. Classen¹. 2010. ¹ Effect of daylength on physiological and behavioral rhythms in broilers. University of Saskatchewan, Saskatoon Canada, ²Aviagen, Huntsville AL. Proceedings of the Poultry Science Annual Meeting, Denver Colorado. Presentation was awarded Graduate Student Certificate for the Behavior and Welfare section.
- K. Schwean-Lardner**, B.I. Fancher and H.L. Classen. 2009. Darkness and the partitioning of darkness – does it affect productivity of commercial broilers? Proceedings of the 8th European Symposium on Poultry Welfare, Cervia, Italy.
- K. Schwean-Lardner**, B.I. Fancher and H.L. Classen. 2009. Darkness and the partitioning of darkness – does it affect the welfare of commercial broilers? Proceedings of the 8th European Symposium on Poultry Welfare, Cervia, Italy.
- K. Schwean-Lardner**, B.I. Fancher and H.L. Classen. 2009. Darkness and the partitioning of darkness – does it affect the behaviour of commercial broilers? Proceedings of the 8th European Symposium on Poultry Welfare, Cervia, Italy.
- K. Schwean-Lardner**, H.L. Classen and B.I. Fancher. 2008. The effect of graded hours of daylength on broiler behaviour. Proceedings of the World's Poultry Congress, Brisbane, Australia.
- Dalal, S., **K. Schwean-Lardner**, B. Laarveld, H.L. Classen and A.G. Van Kessel. 2007. Effect of photoperiod on immune function in broiler chickens. Proceedings of the Poultry Science Association Meeting, San Antonio Texas, p. 142.

- Schwean-Lardner, K.,** H.L. Classen and B.I. Fancher. 2007. Increasing lighting program effects on production characteristics of modern broilers. Proceedings of the Poultry Science Association Meeting, San Antonio Texas, p. 165.
- Schwean-Lardner, K.,** H.L. Classen and B.I. Fancher. 2007. Increasing lighting program effects on production characteristics of modern broilers. Proceedings of the Southern Poultry Science Meeting, Atlanta Georgia.
- Schwean-Lardner, K.,** H.L. Classen and B.I. Fancher. 2006. The effect of daylength on the behaviour of broiler chickens. Proceedings of the Poultry Science Association Annual Meeting, Edmonton, AB, p. 103.
- Schwean-Lardner, K.,** H.L. Classen and B.I. Fancher. 2006. Effect of photoperiod on mobility and leg defects in broilers. Proceedings of the Poultry Science Association Annual Meeting, Edmonton, AB, p. 103.
- Schwean-Lardner, K.,** H.L. Classen and B.I. Fancher., 2006. Daylength effects on production traits of modern broilers. Proceedings of the Southern Poultry Science Meeting, Atlanta Georgia.

8.3 Invited presentations

- K. Schwean-Lardner** and H.L. Classen. 2010. Current understanding of poultry behaviour and handling. Use of behaviour as a tool in the measurement of poultry welfare. A workshop on the welfare of animals at the time of slaughter: A Better Training for Safer Food Initiative of the European Commission/DG-SANCO and CFIA. Calgary, Alberta. Nov 23rd-25th.
- K. Schwean-Lardner. 2010.** Impact of daylength on welfare of broilers. Ministry of Agriculture and Forestry, Government of New Zealand Welfare Codes Committee. Wellington, New Zealand, October 19th.
- K. Schwean-Lardner,** H.L. Classen, B. Fancher, A. Deep and C. Raginski. 2010. University of Saskatchewan – Lighting Research Summary. Tegal Foods, Auckland New Zealand, October 18th.
- K. Schwean-Lardner. 2010.** Impact of daylength on welfare of broilers. Tegal Foods, Auckland New Zealand. October 18th.
- K. Schwean-Lardner. 2010.** Impact of daylength on welfare of broilers. Australasian Poultry Veterinary Conference, Christchurch, New Zealand. October 14th.
- K. Schwean-Lardner. 2010.** Let there be light – and dark! Poultry Service Industry Workshop. Banff, Alberta. October 7th.

- K. Schwean-Lardner. 2010.** Animal welfare and its impact on market access. Prairie Poultry Meeting, Edmonton, AB. June 6th-7th.
- K. Schwean-Lardner**, and H.L. Classen. 2010. Abrupt changes in lighting programs for broilers. Prairie Poultry Meeting, Edmonton, AB. June 6th-7th.
- K. Schwean-Lardner**, H.L. Classen and B.I. Fancher. 2010. Broiler lighting programs and their effects on marketing and welfare traits. Canadian Poultry and Egg Processors Council Annual Meeting, Saskatoon, Canada. June 4th.
- H.L. Classen, **K.V. Schwean-Lardner** and B.I. Fancher, 2010. Broiler Lighting, an Underestimated Management Technique. Poultry Industry Research Day, Guelph Ontario, May 11.
- H.L. Classen, **K.V. Schwean-Lardner** and B.I. Fancher, 2010. Broiler Lighting, an Underestimated Management Technique. 2010 Mid-Atlantic Nutrition Conference Baltimore Maryland, 10 pages, March 24-25.
- K. Schwean-Lardner**, H.L. Classen and B. I Fancher. 2010. The impact of darkness exposure on the welfare and productivity of modern commercial broilers. Aviagen International Webinar Series (3 presentations), Edinburgh Scotland. March 22nd – 24th.
- K. Schwean-Lardner** and H.L. Classen. 2010. Poultry Welfare Issues in Canada: Are we underestimating the importance of light in a broiler flock? World Poultry Science Association German Branch Plenary Speaker, Grevenbroicher, Germany. March 16th.
- K. Schwean-Lardner.** 2010. Why animal welfare impacts market access. Saskatchewan Poultry Industry Conference, Invited Lunch Speaker, Saskatoon Canada. March 4th.
- H.L. Classen, **K.V. Schwean-Lardner** and B.I. Fancher, 2009. Practical Application of Lighting Principles to Broilers. Aviagen Technical Meeting, Huntsville, Alabama, December 1, 2009.
- H.L. Classen* and **K.V. Schwean-Lardner. 2008.** The Impact of Commercial Broiler Lighting Programs and Their Interaction with Nutrient Density (Amino Acids and Energy). Proceedings of the Arkansas Nutrition Conference, 8 pages (CD format), Rogers, Arkansas, September 9.
- H.L. Classen, **K.V. Schwean-Lardner** and B.I. Fancher, 2009. Interactions between **Daylength and Nutrition in Broilers. Informal Nutrition Conference, Poultry Science Association Annual Meeting.** Raleigh North Carolina, July 20.

H.L. Classen, **K.V. Schwean-Lardner** and B.I. Fancher. 2008. Practical application of lighting principles to broilers. Aviagen Technical Meeting, Krakow, Poland, September 22.

H.L. Classen and **K.V. Schwean-Lardner**. 2008. Practical application of lighting principles to broilers. Presented to Cobb-Vantress, Incorporated, Siloam Springs, Arkansas, September 8.

Schwean-Lardner, K. 2007. What happens when the lights go out? Poultry Service Industry Workshop, Banff, AB.

8.4 Technical documents

K. Schwean-Lardner and H.L. Classen. 2010. Lighting for Broilers. Aviagen™ Technical Bulletin, 38 pages (<http://www.rossbreeders.com/ss/technical-center/>), Huntsville, Alabama.

K. Schwean-Lardner. 2010. Lighting for Broilers. Aviagen™ Technical video. (<http://www.aviagen.com/tech-centre/>).

8.5 Upcoming invited presentations

K. Schwean-Lardner, 2012. Photo-periodism in broilers. World Poultry Congress, Salvador Brazil, August.

Chapter 9. Appendix

Table not published. The effect of daylength on the percentage of bone ash.

	Daylength (h)				Pooled SEM ¹	P value ²	Equation
	14	17	20	23			
<u>31 days</u>							
Bone ash, %	52.55	52.02	51.24	51.52	0.123	0.0393	$Y=61.596993-0.954642x+0.022342x^2$
<u>38 days</u>							
Bone ash, %	42.69	43.36	43.71	42.56	0.805	NS	-
<u>49 days</u>							
Bone ash, %	32.48	33.84	34.89	36.02	0.427	0.0012	$Y=27.10888+0.38896x$

Values listed for lighting program means and for Standard Error of Means (SEM) and regression equation calculation based on original data.

^{A, B} Means with common letters do not differ significantly (P<0.05)

¹ SEM – Standard error of the mean

² Values for the P (probability of regression) and R² values based on log transformed values.

Table not published. Effect of gender and genotype on bone ash – Experiment 1

	Gender		Genotype		Light x Gender	Light x Genotype	Gender x Genotype	Light x Gender x Genotype
	Male	Female	308	708				
<u>31 d</u>								
% tibia wt	51.59 ^B	52.08 ^A	51.79	51.88	NS	NS	NS	NS
<u>38 d</u>								
% tibia wt	43.09	43.07	43.42	42.74	NS	NS	NS	NS
<u>49 d</u>								
% tibia wt	34.04	34.57	34.47	34.14	NS	NS	NS	NS

^{A, B} Means with common letters do not differ significantly ($P < 0.05$).

Table not published. Effect of gender x genotype on “other” cause of mortality and culls (% of birds placed) 7 to 48 days of age – Experiment 1

	Gender	
	Male	Female
308	0	0.59
708	0.99	0.09

Table not published. Effect of gender x lighting program on gait score category 4 – 28 d – Experiment 1

	Gender	
	Male	Female
14L:10D	0	0
17L:7D	0	0
20L:4D	1.56	0
23L:1D	4.69	0

Table not published. Effect of gender x lighting program on gait score categories 3+4+5 – 28 d – Experiment 1

	Gender	
	Male	Female
14L:10D	0	0
17L:7D	1.56	3.13
20L:4D	4.69	0
23L:1D	9.38	0

Table not published. Effect of genotype x lighting program on gait score category 2 – 35 d – Experiment 1

	Genotype	
	Ross x Ross 308	Ross x Ross 708
14L:10D	1.88	0.63
17L:7D	1.88	1.25
20L:4D	1.88	3.13
23L:1D	3.33	8.00

Table not published. Effect of genotype x lighting program on gait score categories 3+4+5 – 35 d – Experiment 1

	Genotype	
	Ross x Ross 308	Ross x Ross 708
14L:10D	0	0
17L:7D	0.63	0.63
20L:4D	3.75	1.25
23L:1D	7.33	1.33

Table not published. Effect of gender x genotype gait score categories 3+4+5 – 49d – Experiment 1

Gender		
	Male	Female
308	12.83	6.41
708	14.19	5.30

Table not published. Effect of sex x lighting program on category 2 footpad scores– 28 d – Experiment 1

Genotype		
	Male	Female
14L:10D	1.56	4.52
17L:7D	3.13	9.38
20L:4D	6.25	4.69
23L:1D	12.50	4.92

Table not published. Interaction of lighting program and genotype ocular weight (% of live weight) – 31 d – Experiment 1

	Ross x Ross 308	Ross x Ross 708
14L:10D	0.20	0.20
17L:7D	0.18	0.20
20L:4D	0.18	0.20
23L:1D	0.21	0.25

Table not published. Interaction of lighting program and genotype ocular weight (g) – 46 d – Experiment 1

	Ross x Ross 308	Ross x Ross 708
14L	4.79	4.80
17L	4.95	4.75
20L	4.75	4.78
23L	5.33	5.43