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Faculty of Veterinary Medicine and Animal Sciences

Optimal Light Spectrum to Laying Hens

R.M.K. de Groot

Department of Animal Environment and Health

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R.M.K. de Groot

Supervisor: Wichman, A.
Department: SLU; Department of Animal Environment and Health

Assistant Supervisor: Rubene, D.
Department: SLU, Department of Ecology

Examiner: Yngvesson, J.
Department: SLU, Department of Animal Environment and Health

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Abstract

Modern poultry farming still struggles with high levels of feather pecking in laying hens *(Gallus gallus domesticus)*. This form of abnormal pecking behavior is known to be affected by multiple factors, including effects from the light environment. Illumination in poultry housing is in many ways different from the light environment from the ancestor of the chicken – a species that has one of the most advanced visual systems and is able to see into the ultraviolet range. This study performed two experiments using three light treatments: Jungle light (imitating light in the jungle), D65 light (a standardized daylight illuminant) and control (commercial standard). Jungle light and D65 light included ultraviolet, while control light did not. During the home pen observations occurrences of behavior and social behavior were measured and compared between the three light treatments. No differences in behavior observed were significant, but there was a trend for more stretching behavior in D65 light. Age and time of day affected a couple of behaviors. In the preference tests hens were able to choose between two types of light during a three day period. A tendency for overall preference for the different light treatments was found. Laying hens preferred jungle over control light, and there was a trend for D65 over control light. There still seems to be a preference from laying hens for light spectra that imitate the light environment of their ancestors, including ultraviolet, compared to light conditions in modern poultry housing.

Keywords: laying hen, behavior, light, spectrum, preference

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1 Introduction

Poultry farming is an important part of food production systems. In Western Europe, within the last decades poultry farming has become an intensified, modern and highly specialized farming system, consisting of a separate broiler and laying hen industry. The laying hen industry has undergone revolutionary change in the European Union during the start of the 21st century, as battery cages have been banned and new housing systems have been developed (EC no. 74/1999). Ongoing developments in both high quality nutrition and advanced genetics enabled the industry to rapidly increase production levels in both layers and broilers *(Gallus gallus domesticus)*.

Despite increasing levels of production, there are still a lot of problems within both sectors. In the cageless laying hen industry one of the major problems for many years has been feather pecking, with an estimated prevalence of 40 – 80% (Blokhuis *et al.*, 2007). Feather pecking and cannibalism cause concern from both welfare as economic standpoints (Savory, 1995). Research has shown that this problem behavior is not easily solvable due to its multi-factorial nature – genetics, feed and environment all play their part (Blokhuis, 1986; Kjaer & Sørensen, 1997; Wahlström *et al*., 1998). One of the several suspected causes of feather pecking behaviors is the lighting in the production environment, which is in many ways different from the natural light found in the forests where ancestors of our modern poultry used to roam around (Prescott *et al*., 2003). Not all differences in the light environment are perceived by human as fowl have more refined vision compared to humankind (Bowmaker *et al*., 1997).

In the current study behavior will be compared for laying hens housed in three different light treatments. Two of these light treatments imitate types of the natural environment of the ancestor of the modern chicken (jungle light and D65), the other represents the commercial standard (control).

1.1 Bird vision

The eye of the bird resembles that of other vertebrates in general structure. Yet, the bird's eye is relatively larger, the shape more spherical and the eye is far less mobile. Instead of turning their eyes, birds are able to scan the visual field by turning their heads into almost every direction. Additionally bird eyes, like other vertebrates but not mammals, are equipped with a sclerotic ring. The plate like bones that form this ring do not only give strength, but help focus the large eye as well. The pecten is another distinctive feature of the bird's eye. The pecten is a highly vascularized organ which is attached to the retina, located close to the optic nerve. It is hypothesized that the function of the pecten would be to provide nourishment to the retina (Hickman *et al*., 2010).

The color vision of chickens and other birds is different from human in several aspects. The visual system of birds contain complex structures that allow for a more refined vision compared to human, and probably most other species. As Bowmaker et al. (1997) states: "Diurnal neognathus birds probably have, at least at the retinal level, one of the most elaborate mechanisms for color vision within the vertebrates." While human possess only two types of photoreceptors, rods and cones, birds possess an additional third type of photoreceptor, a double cone. Though the function is still debated, it is thought that double cones play a role in achromatic tasks, such as the detection of movement and recognizing patterns (Hart & Hunt, 2007). Cones, playing an important role in color vision, are more numerous compared to rods in diurnal birds like the chicken (Hickman *et al*., 2010).

Human color vision is based on three cone cell species, while fowl, like other birds, possess four different types of cones, making them tetra chromatic. Yoshiziwa (1992) found the peak absorptions to occur at the values of 415 nm (chicken violet), 455 nm (chicken blue), 508 nm (chicken green) and 571 nm (chicken red). Behavioral studies have confirmed the difference in spectral sensitivity between chicken from human and shown that chicken can perceive the ultraviolet range (400 nm and lower) indeed (Prescott & Wathes, 1999). Another feature that distinguishes avian from mammalian vision, is the presence of oil droplets. These oil droplets filter incident light before reaching the photoreceptors. Five different types of oil droplets have been identified, of which most were associated with the single cone cell type (Bowmaker & Knowles, 1977). The pigmented oil droplet in front of a cone pigment restricts the sensitivity of the receptor to a more narrow bandwidth, which is suggested to lead to fine tuning of hue discrimination (Partridge, 1989).

1.2 Effects of the light environment

Natural light consists of two elements: Direct sunlight and diffuse light reflected from other surfaces. The light environment in poultry production systems are generally controlled by artificial lightning, and little natural daylight. These artificial light circumstances are in many ways different to the light environment in the natural habitat, which avian vision is presumed to be optimally adapted to (Prescott *et al*., 2003). Light in the natural environment consists of a broader light spectrum including UV, with varying light spectrum and intensity in different parts of the habitat and at different times of day. Forests exhibit much variation in light environment, depending on forest structure, weather conditions and time of day (Endler, 1993).

Controlled photoperiod in the laying hen industry is often very different from photoperiod encountered naturally as changes in the dark : light ratio are used to commence laying period or initiate sexual maturity (Sharp, 1993). During rearing photoperiod plays an important role in retinal development, where continuous lighting programs result in damage to the hens' eyes (Li *et al*., 1995). Furthermore the flickering of artificial lighting gained attention as it may be perceived by domestic chickens who have been found to have a higher temporal resolution compared to human (Jarvis *et al*., 2002; Railton *et al*., 2009). In the last fifty years two aspects of lighting have received great interest from researchers worldwide as they are suspected to influence behavior in chicken: light intensity and light spectrum.

1.2.1 Intensity

In general, it is thought that reducing light intensity decreases overall activity, aggressive behavior and cannibalism (Hughes & Duncan, 1972; Hughes & Black, 1974; Kjaer & Vestergaard, 1999; Deep *et al*., 2013). Yet several studies have been done which do not confirm this hypothesis (Hartini *et al*., 2002; Kjaer & Sørensen, 2002; Shinmura *et al*., 2006). Furthermore, age seems to affect the relation between light intensity and behavior (Davis *et al*., 1999; Kristensen *et al*., 2007). When given the choice, both broilers and laying hens chose to spend most time at bright light conditions at two weeks of age, though changed their preference to dim light at six weeks of age (Davis *et al*., 1999). Accordingly, birds of one week of age dust-bathed more at bright light conditions, whereas at six weeks of age dust-bathing took place most at dim light conditions (Kristensen *et al*., 2007). It should be noted that these findings contradicts earlier research about bird species – including hens – being attracted to, and sun-bathe in bright light conditions (Manser, 1996).

Light intensity can affect the social communication between hens, but only when reduced extremely. Kristensen et al. (2009) showed an effect in the social communication between hens competing for food when light intensity was as low as 1 lux. Light intensities of 5 lux and higher did not interfere with the social communication patterns. On a physiological level, it has been shown that light intensities as low as 1 lux reduce egg production and laying sequence length. Remarkably, in the same study it was found that the brightest intensity of 500 lux affected egg production negatively as well. The brown breeds were more strongly affected than the white ones, suggesting that genotype plays a role in susceptibility to the negative effects of low or high light intensity (Renema *et al*., 2001). The relationship between light intensity and feather pecking was suspected to be affected by breed as well by Kjaer & Sørensen (2002). For broilers a minimum threshold of 5 lux has been determined to prevent from negative effects on both welfare and performance (Deep *et al*., 2013).

1.2.2 Spectrum

Spectrum has been found to affect chicken as early as during the embryonic stage. Wavelength specificity has been shown for the development of the structural and functional asymmetry, which plays an important role in avian visual behavior (Rogers & Krebs, 1996). Another recent discovery is a magnetic compass chicken use for orientation, which has been found to be wavelength dependent. When tested under monochromatic light, young domestic chickens were able to orientate under blue, but not red light (Wiltschko *et al*., 2007). Significant differences in body weight, bone strength and lameness problems have been found in experiments with colored light, yet they might all be direct consequences of differences in behavior (Prayitno *et al*., 1997a; Prayitno *et al*., 1997b).

Thus far it seems that blue and green light have a calming effect on chickens whilst red light increases activity (Prayitno *et al*., 1997a; Prayitno *et al*., 1997b; Sultana *et al*., 2013). Tonic immobility testing, used as an indication of fear, showed significantly higher results for broilers raised under red light compared to those under blue light (Sultana *et al*., 2013). Blue light is also the preferred option when birds are given the possibility to choose between blue, green, red or white. Interestingly this preference was found for broilers raised in red, white and green light, yet the birds raised in blue had a preference for green light (Praytino *et al*., 1997a). In an earlier study by Cave (1990) green light was found to reduce mortality and increase fertility compared to white light. Effects on laying performance, such as egg production and egg weight, were not proven. Neither was a difference in growth rate or food intake of broilers from either sex (Wathes *et al*., 1982).

It is becoming increasingly clear that ultraviolet light plays an important role in avian color perception and affects bird behavior (reviewed in Cuthill *et al*., 2000). Furthermore, the presence of UV wavelengths was found to improve the temporal resolution in chickens (Rubene *et al*., 2010). Research shows that even for domestic chickens ultraviolet could be of importance to obtain good welfare. Rearing layer chicks under UV deficient environments was found to result in consistently higher basal plasma corticosterone concentrations and a trend of less exploratory behavior. As increased plasma corticosterone levels in birds are associated with chronic stress, decreased welfare is implied (Maddocks *et al*., 2001). Additionally, ultraviolet light was found to play a role in the transmission of sexual signals or communication in broilers – its absence could impair the welfare of the breeder birds (Jones *et al*., 2001).

Light source is often a determining factor for the wavelength emitted in the environment of chickens. Standard artificial light sources produce little, if any, ultraviolet radiation. A preference for fluorescent light compared to incandescent light has been found both in layers as in broilers (Widowski *et al*., 1992; Kristensen *et al.*, 2007) It has been suggested that this preference could be due to the more blue wavelengths of the fluorescent light (Widowski *et al*., 1992). Additionally, it was found that next to warm white fluorescent light, also biolux light was a preferred option, while spectral sensitivity light was not. Interestingly, the preferred sources of warm white fluorescent and biolux light (similar colour temperature to daylight) were the closest to daylight in spectral composition, while the spectral sensitivity sources (light made up to follow the spectral sensitivity of fowl) and incandescent sources were more biased towards specific parts of the spectrum (Kristensen *et al*., 2007).

Next to preference, these light source studies described some behavioral findings as well. Laying hens showed more preening behavior in fluorescent light compared to incandescent light (Widowski *et al*., 1992) and broilers performed less feather and object pecking behavior in warm-white rather than biolux light (Kristensen *et al*., 2007). Performance was not affected by light source when fluorescent and incandescent light were compared in broilers (Scheideler, 1990; Buyse *et al*., 1996). However, more elaborate studies with various light sources have found differences in broiler performance, though the results were not consistent (Zimmerman, 1988).

1.3 Chicken behavior

When modern day chickens are provided with adequate space and resources, their normal behavioral repertoire comprises ancestral behavioral patterns (Lay *et al*., 2011). The extent to which expression of these behavioral patterns takes place depends on many factors. Besides their direct environment and previous experiences; genetics, epigenetics and environmental conditions during embryonic development all impact adult behavior (Janczak *et al*., 2007). Furthermore time of day and age are known to affect behavior in chicken (Savory & Maros, 1993).

In 1964, Kruijt performed a large behavioral study on the ancestor of the modern chicken (*Gallus gallus domesticus*); the red jungle fowl (*Gallus gallus spadiceus*). In his work he makes a distinction between social and maintenance behaviors.

Maintenance behaviors are those behaviors without a social function, such as locomotion, feeding and drinking and comfort behavior. He found that in the first two weeks of life, almost all behavioral patterns without social function are developed. The locomotory behaviors of fowl consist of walking, running, jumping, hopping, wing movements and flying. Ground-pecking behavior starts directly after hatching (Kruijt, 1964). Pecking in the feeding manner at all sorts of objects is a common observation for all birds of the family of the chicken (Galliformes). It is through this trial-and-error pecking that chickens become acquainted to a wide variety of possible food sources. This behavior that cannot be changed by a sudden change in environment or feeding conditions, is seen as an instinctive behavior (Hoffmeyer, 1969).

Feeding activities are more than ground pecking alone. Other behaviors serve to make the food more accessible or more easily edible. Kruijt (1964) described these feeding activities as ground-scratching, head-shaking, bill-wiping, bill-beating, head-scratching and food-running. Drinking behavior is characterized by the swallowing movement, for which the bird keeps the neck in outstretched position and raises the head. Furthermore he defined activities that are associated with care of the body and body surface as comfort behaviors. Those behaviors can be divided into stretching, preening, dust bathing, and shaking (Kruijt, 1964).

1.3.1 Social behavior

Behavior that appears to be related, causally and functionally, to other individuals of the same species is defined as social behavior (Kruijt, 1964). The jungle fowl have been found to be a harem living polygynous species and the females in each flock form their own dominance hierarchy. Female to female dominance relationships have often been reported to be extremely stable, much more than between males (reviewed in Keeling & Gonyou, 2001). In caged laying hens, an increase of group size leads to increased agonistic behaviors and higher pecking damage (Hughes & Duncan, 1972; Al-Rawi & Craig, 1975). It is generally assumed to be unlikely that birds in industrial-sized flocks are able to form a hierarchy (Keeling & Gonyou, 2001). Pagel and Dawkins (1997) described a model suggesting laying hens in large flocks using social signals of status, rather than attempting to establish a peck order.

Communication takes primarily place through signals provided by postures, displays and vocalization, which can signal threat and submission (Kruijt, 1964). Visual discrimination of individual birds and recognizing postures and status signals implies the importance of light environment for social behavior (Kristensen *et al*., 2009). Besides visual cues, domestic chickens use dozens of different vocalizations, which can be roughly categorized into calls of warning, reinstatement and contact calls, territorial calls, mating calls, laying calls and food calls (Keeling & Gonyou, 2001).

1.3.2 Abnormal behavior

Generally behaviors are considered abnormal when not encountered in the wild population or when either caused by an underlying pathology or causing pathology (Mason, 1991). The abnormal behavior encountered most in laying hens are feather pecking and cannibalism. The pecking and pulling of feather from another bird is defined as feather pecking, while cannibalism includes the pecking and tearing of another bird's skin and underlying tissues (Keeling & Gonyou, 2001) However, not all feather directed pecking should be seen as abnormal or as a form of aggression, since fowl also use more gentle forms of pecking in their social communication. Gentle pecks are most often seen in the context of dust-bathing, while aggressive pecks occur regularly in the context of feeding (Leonard *et al*., 1995). Cannibalism often results in serious injury and death and usually follows from feather pecking (Hughes & Duncan, 1972).

A special form of cannibalism is vent pecking – directed towards the bird' s cloaca - which generally occurs soon after hens come into lay (Savory, 1995). Vent pecking, also known as cloacal cannibalism, seems to be unrelated to aggressive pecking on other parts of the body (Gunnarsson *et al*., 1999)

1.4 Preference testing

Using motivation and preference tests to investigate behavioral needs has become a very influential technique to assess animal welfare. By giving animals some control over their environments and observing the choices being made, subjective experiences of animals become measurable. The difference between the strength of motivation to achieve or avoid one resource or stimulus and the strength of motivation to achieve or avoid another is denoted as preference. Preference is not the same as choice, as choice describes instances of behavior, while preference describes characteristics of animals. For example 'the chicken chose to eat a mealworm' versus 'this chicken prefers mealworms over seeds' (Kirkden & Pajor, 2006).

If more than two stimuli are to be tested, a choice must be made between simultaneous and successive testing. Simultaneous presentation of test stimuli might be distracting but allows the animal to make simultaneous comparisons between them. However, successive tests could become confounded by order effects as a result of reduced responsiveness during the course of testing. A problem that might occur with the method of preference testing is that a subject may become 'trapped' by the first option it chooses. This could be simply because the subject happened to face one direction and approaches that option first. Another problem can be the incorrect interpretation of withdrawal from one option as preference for the other option (Martin & Bateson, 2007). Still, preference testing is a popular option as it is one of the few methods which allows subjective experiences of animals to become something measurable.

1.5 Aim

This study is part of a project aiming to find the optimal light conditions for laying hens that suit their visual system. These conditions should allow hens good opportunities to forage, recognize other hens and move around securely and with precision, while minimizing both stress and the development of problem behaviors.

The specific study presented in this degree project aims to find the optimal light spectrum to laying hens. The research questions are to:

1. Find if laying hens have a preference for light spectrum that imitates the natural light environment of their ancestors.
2. Assess if behavior is affected by spectral composition of light.
3. Discover if light spectrum during rearing impacts preference for light environment and behavior later in life.

It is hypothesized that laying hens have a preference for light spectrum similar to that of the natural environment of their ancestors, for behavior to be affected by spectral composition and for light spectrum at young age to affect spectral preference and behavior later on.

2 Materials & Methods

For this experiment laying hens of the breed Bovan Robust were used. All hens were ordered from the same company and raised and housed in similar circumstances, except for the difference in lighting between groups.

2.1 Housing conditions

The pens measured 3.85 m × 3.62 m, and consisted therefore of 13.9 m2, of which about one third (4.7 m2) was litter area. All 12 pens were of identical measurements and included the following attributes: A perch, a drinking bowl and two feeding trays, see figure 1. Feed and water was provided ad libitum. Separations between pens existed in the form of metal grid and dark grey tarpaulin, to prevent interference from light sources from adjacent pens. The front separation was built of metal grid only, meaning light from opposing pens could reach each other. Therefore opposing pens usually had the same type of test light, or if not, an extra separation of tarpaulin was placed in between. The tarpaulin separations could not be placed in such a manner that all interfering light was blocked completely, but prevented to such extend that interference on light spectra was not measured in detectable amounts by the spectrophotometer. All pens faced the outer wall at the backside, made of concrete and partially covered with a heating element. The permanent window and outdoor accesses in the back walls were closed, to prevent natural light from interfering with the test light.

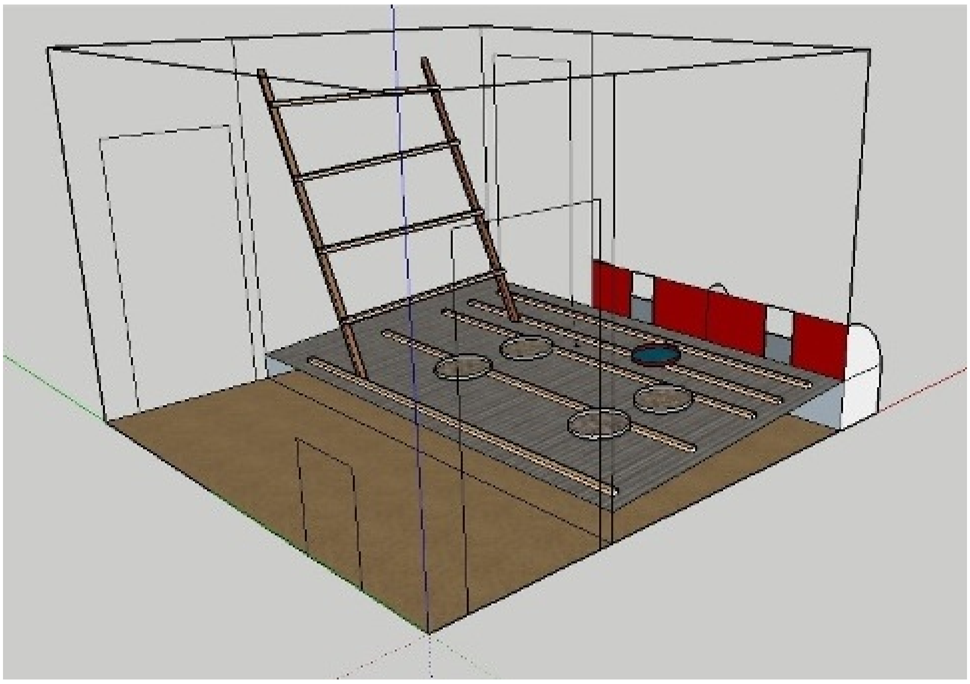

Figure 1. Scaled impression of the home pen.

2.1.1 Rearing

Hundred and ninety-two day-old chicks arrived on the experimental farm and were divided into six groups of 32 chicks which were placed into the litter area of their home pens. The chicks had already been vaccinated against Mareks disease before transport. The litter area was covered with wood shavings and unlimited feed and drinking water was provided in trays adapted to young hens. The slatted floor was not made accessible yet, as this was considered unsuitable to very small chicks. The temperature in the stable was started at 23 to 24 degrees Celsius, and gradually decreased to 20 degrees Celsius in week five. Heat lamps were provided until week five and provided temperatures around 37 to 39 degrees Celsius.

One week after arrival all chicks were vaccinated against coccidiosis through the drinking water. After 18 days the separation between litter and slatted floor area was taken away which gave the chicks access to the full pen. In week five of the experiment, each group of 32 was split into two groups of 16, filling 12 pens in total. In the first three weeks the chicks were visited by one of the people involved in the project on a daily basis, for purposes of habituation. Photoperiod was started with 23 hours of light and gradually decreased to 10 hours of light in week seven (from 07:00 till 17:00), after which it remained constant until week fifteen. During week fifteen and week sixteen, photoperiod was increased up to 12 hours (from 6:00 till 18:00), after which it remained constant until the end of the trial. Nest boxes were closed until start of lay, at week 17.

2.1.2 Lighting treatments

Three types of lighting treatment were used which were named control light, D65 and jungle light. The control light represented the commercial standard and its wavelength ranged from 415 to 700 nm, with a sharp and a broad peak, at 445 nm (blue) and 611 nm (green-red) respectively, see figure 2. D65 is a standardized illuminant for daylight which was similar to the light measured at forest edges where jungle fowl usually forage. The spectral composition of this light consisted of two sharp peaks at 397 nm (UV) and 450 nm (blue), plus a very broad peak with its summit about 595 nm (green-red), see figure 3. Jungle light imitated the light as measured in the forest interior, where jungle fowl commonly roost. The radiated light from the jungle lamps consisted of several sharp and semi-sharp peaks, occurring at the wavelengths of 400 nm (UV), 450 nm (blue), 513 nm (green) and 620 nm (red), see figure 4. During the experiment, the average intensities for control, D65 and jungle light in the home pens were measured at 7,0, 11,0 and 8,0 lux respectively.

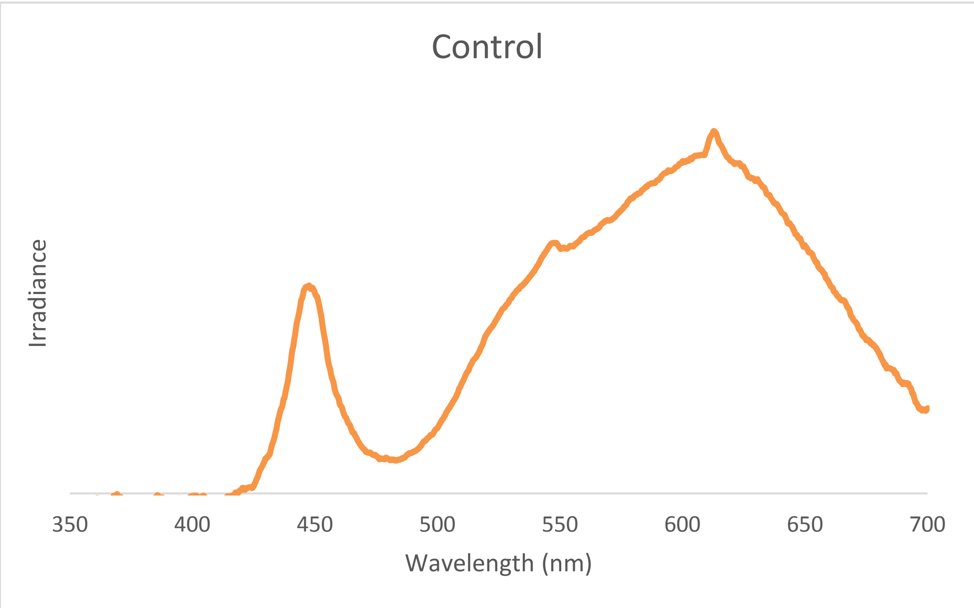

Figure 2. Spectral composition of control light.

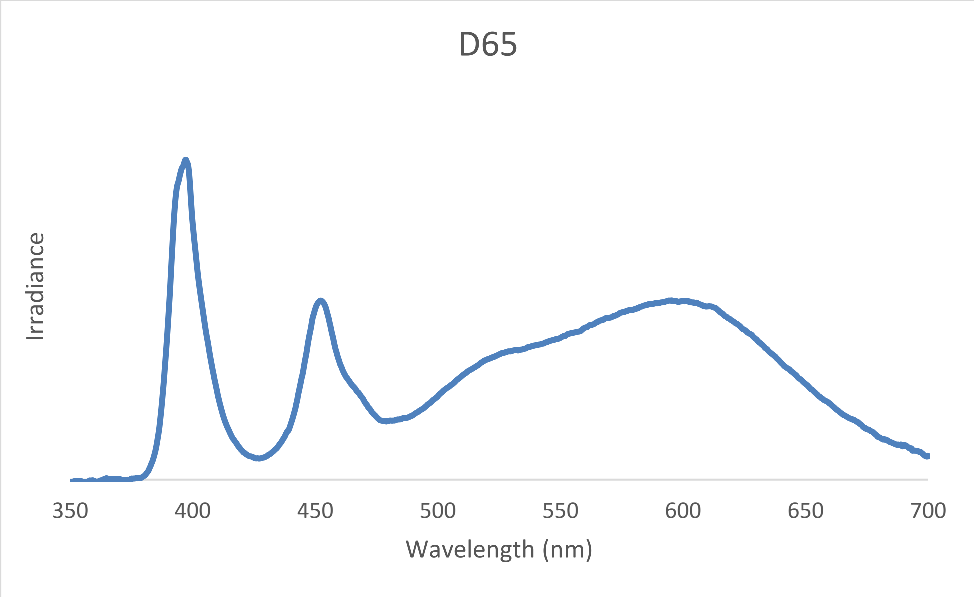

Figure 3. Spectral composition of D65 light.

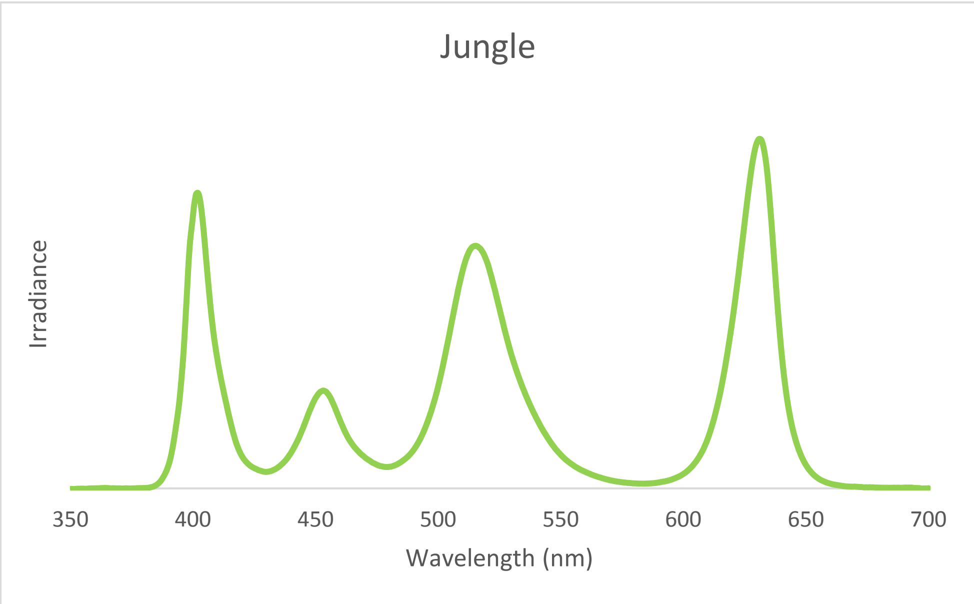

Figure 4. Spectral composition of jungle light.

In week 5 of the experiment, all test lights were placed. Before placing the test lights all hens were reared in Control light. The duration of day length was gradually built up as to simulate spring and to stimulate the start of the laying cycle. The exposure time to the light was controlled by individual manual timers for both the jungle light and D65, but control light was set by an automatic system. Changes from darkness to artificial daylight and vice versa were abrupt for the manually controlled jungle light and D65, but gradually changed over a time span of 15 minutes for the control light. As the timers were not extremely precise, all lights in the stable did not turn on and off at the exact same time, which provided a dimming effect in all pens.

2.1.3 Spatial planning

The stable gave room to eighteen pens, for which twelve were used as home pens in our experiment. Of these twelve pens, four were illuminated with control light, four with jungle light and four with D65. The different lighting treatments were designated as balanced as possible, in such a manner that two adjacent pens were never illuminated by the same type of light. Three pens were occupied for another part of the same project, and the three last pens were designated as preference pens. Before the hens moved into the pens, light intensity was measured and if necessary adjusted in all pens. Laying hens that needed to be isolated or tested for other experiments, were taken outside this stable section and brought into another section of the building.

2.2 Home pen observations

From week five, behavioral observations were executed about once every three to four weeks, until week 20. These home pen observations were a combination of scan sampling and continuous sampling. Observations took place for each group twice; in the morning and in the afternoon, often on the same day, but sometimes a few days apart from each other. Especially when the preference tests had started, performing home pen observations on all groups in one day was not possible due to three groups being located in the preference pens. The observations were carried out at five different ages; at 6, 8, 12, 17 and 21 weeks of age, executed by two different observers. The ethogram and protocols were tested and improved by one of the observers before the first home pen observations took place. At the start of the observations the two main observers scored a few pens simultaneously to compare the similarity of their outcomes.

2.2.1 Sampling method

Observers were standing in front of the pens, and were visible to the hens. One minute of adjustment time was given before starting scan sampling, whether the hens were still affected by the observer or not. After the first scan sample, the ten minutes of continuous sampling followed immediately. Halfway through the continuous sampling – after five minutes – another scan sample was taken. At the end of the ten minutes of continuous sampling a last scan sample took place. Therefore each observation resulted in three scan samples and two five minute periods of continuous sampling. There were 10 categories used in the scan sampling, which were mutually exclusive from each other. These categories were defined as drink, feed, stand, locomotion, forage, rest, perch, preen, dust-bathe and other. The category other included out of sight. When a laying hen was located on the perch, this overruled other types of behavior, such as preen or locomotion, and a score for perch was given. For ethogram see table one. During the continuous sampling eight categories were considered, with a focus on social behavior: Aggression, severe peck, gentle peck, wing-shake, stretch, run, sparring and vocalize. If any of those behaviors were performed during the sampling period, each occurrence was scored. For ethogram see table two.

Table 1. *Descriptions of all observed behaviors during scan sampling home pen observations*

Behaviour	**Description of behaviour**
Drink	Beak in or above drinker
Feed	Beak in or above feeder
Stand	The abdomen is not touching the litter + the hen is motionless
Locomotion	Moving around
Forage	Pecking or scratching the litter
Rest	Resting abdomen on the litter
Perch	Located on the perch
Preen	Beak touches plumage of hen itself
Dust-bathe	In a sitting or lying position, flapping wings in substrate, partly burying body in substrate
Other	All other behaviour not identified above, including out of sight

Table 2. *Descriptions of all observed behaviors during continuous sampling home pen observations*

Behaviour	**Description of behaviour**
Aggression	Aggression. Frontal displays with raised hackles towards other hens , head pecking, jumping or kicking at other hen
Severe pecking	Severe pecking at other hen
Gentle pecking	Gentle pecking at other hen
Stretch	Either wing or leg is lifted off ground and away from body as far as possible
Run	Moving around in a fast manner, including airborne moments
Sparring	Playful frontal displays, often accompanied with little jumps
Vocalize	Semi-loud or loud vocal sound produced by hen

2.3 Preference testing

Preference testing was started at week 16 and lasted until week 24. All groups were exposed to the preference testing three times, with around four weeks between tests. The preference was paired so that after three times each group had been in combination of each pair of lights; that is to say control vs D65, control vs jungle and D65 vs jungle. Each group was tested in one of the three preference pens once, so that no group was in the same pen twice. The twelve groups were divided into four blocks of three groups, and groups of one block would always be tested simultaneously. The groups were designated into the blocks as balanced as possible and always contained one group of each different type of lighting in the home pens. Each type of light was tested equally much on the left as on the right side of the pens and each type of light was equally much tested in each of the three pens.

2.3.1 Catching and moving procedure

The hens were moved from their home pens into the preference pens, where they stayed for at least three days of observations before moving back to their home pens. Moving was facilitated by large plastic containers with a cardboard lid, which allowed for ventilation. Some wood shavings was placed on the bottom. Hens were caught individually and handled carefully into the containers. A maximum of four hens were put into the containers at once. During the catching procedure all lights were turned off, which made the hens easier to catch and seemed to reduce the amount of stress involved. The containers were carried manually from one pen to another and upon arrival in the test pens, hens were released immediately.

2.3.2 Preference pens

The preference pens were of the same size as the home pens, with the same proportions of litter and slatted floor areas. In the middle of the pen a large sheet of tarpaulin was placed from the roof down until 30 cm above the floor, dividing the pen into two equal light areas while allowing the laying hens to move from one section to another, see figure five. On each side a perch, nest box, drinker and feeder were placed, which mirrored the drinker, feeder, nest box and perch on the opposing side. As in the home pens, drink and feed was provided ad libitum. The perches were constructed in such a way that two 1.20 m wide sticks were available at the height of approximately 0.5 m and 1 m. The perch at each side would be able to fit all sixteen hens at the same time, if demanded. Nest boxes were 51.5 cm long × 40.5 cm high × 30.5 cm wide and were manually fabricated out of cardboard moving boxes. On each short side an entrance was made, to enable fleeing behavior from other entering hens.

After each testing period the preference pens were tidied before a new group moved in. All litter material was removed and new wood shavings provided. Depending on the current state of damage, the cardboard nest boxes were (partially) replaced. If necessary, the lights were changed according to the testing schedule. Intensity was measured, and if needed adjusted by placing more or less filter sheets (white diffusion, Lee filters) to cover the lamps.

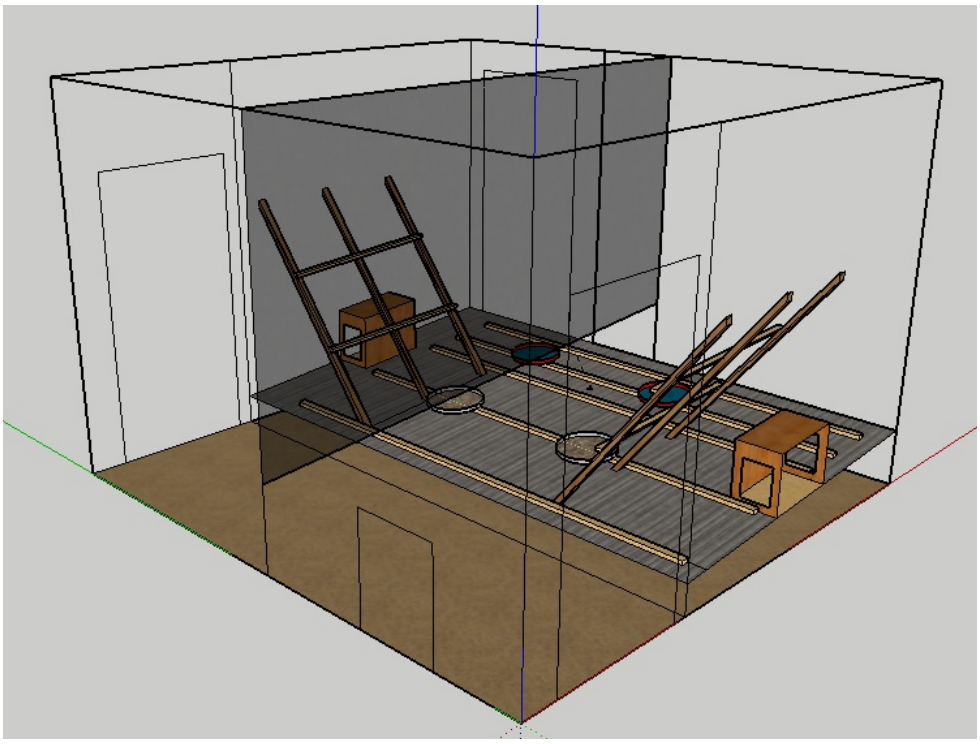

Figure 5. Scaled impression of the preference pen.

2.3.3 Sampling method

The observations of preference were executed as scan samples, taking place every five minutes during a half an hour period. Observations were made four times a day, evenly distributed over the day. The observation periods started at 8:30, 11:30, 14:30 and 17:30. The ethogram and protocol looked slightly different from the home pen observations (see table three), as the protocol was divided into sections representing three areas: Litter, slats and perch. Each area was divided into multiple behavioral categories. Hens on the slats could be scored as drink, feed, stand, locomotion, rest, preen or other. Hens in the littered area were categorized as stand, locomotion, rest, preen, dust-bathe, forage or other. On the perch the possibilities were stand, locomotion, rest, preen or other. One laying hen should be scored in one area only, which could be challenging when the hens were moving around a lot.

Table 3. *Descriptions of all observed behaviors during scan sampling preference tests*

Behaviour	**Description of behaviour**
Located at slats;	
Drink	Beak in or above drinker
Feed	Beak in or above feeder
Stand	The abdomen is not touching the floor + the hen is motionless
Locomotion	Moving around
Rest	Resting abdomen on the floor
Preen	Beak touches plumage of hen itself
Other	All other behaviour not identified above, including out of sight
Located at litter;	
Stand	The abdomen is not touching the floor + the hen is motionless
Locomotion	Moving around
Forage	Pecking or scratching the litter
Rest	Resting abdomen on the floor
Preen	Beak touches plumage of hen itself
Dust-bathe	In a sitting or lying position, flapping wings in substrate, partly burying body in substrate
Other	All other behaviour not identified above, including out of sight
Located at perch;	
Stand	The abdomen is not touching the floor + the hen is motionless
Locomotion	Moving around
Rest	Resting abdomen on the floor
Preen	Beak touches plumage of hen itself
Other	All other behaviour not identified above, including out of sight

A camera (ARLO, Netgear surveillance system) was attached to the ceiling of each half of the preference pens. Behavioral observations were carried out by live observations from the video system, by video recordings or through direct observations in the stable. Most of the observations took place live through the camera system. At the start of the experiment only one screen could be monitored at the time, which resulted in the left side and the right side of the pens, not being scored at the exact same moment. However, later on it became possible to run both screens – left and right – simultaneously. In the few occasions that observations through the cameras was not possible and observations were made directly in the stable, some problems occurred. Some hens were distracted by the observer standing in front of the pens. However, the biggest problem was that it could be hard to see what was going on in the litter area.

2.3.4 Monitoring of wounds

The caretakers at the Lövsta laying hen facility made daily checks on the hens. If any of the hens turned out to be injured, this was noted in the diary. If necessary, hens were taken out of their groups and moved into another section to recover. During our six months experiment, one time a laying hen had to be isolated. Other measures the staff could take in case of wounds was to spray No Fight against further pecking. Mortality was 2%, in total four hens died during the experiment, of which two were found dead before splitting groups took place at week five.

2.4 Statistical analysis

Mean values were calculated for occupancy (preference tests only) and occurrences of behavior during the both experiments. Some behaviors were grouped together for analytic purposes, such as active behavior and comfort behavior in the scan sampling and social active behavior and comfort behavior in continuous sampling. Differences in occurrences for each behavior in both experiments were tested for normal distribution by the use of Shapiro-Wilk tests and QQ-plots. The home pen observation data was not normally distributed and therefore non parametric tests were used, both Kruskal-Wallis (light source and age) and Mann-Whitney (time). These analyses were performed using the IBM SPSS Statistics, version 17.0 software.

Due to the size of the project and the complexity of the statistics it was decided to consider mainly the descriptive data for the preference tests and not run tests for the behaviors. Nonetheless, overall preference and preference in relation to home light was analyzed by general linear modelling (Glimmix) using a binomial distribution. Post-hoc difference was investigated with least square means and an adjustment for multiple comparisons by Tukey-Kramer method. This analysis was performed using software from SAS Institute Inc.

3 Results

Generally laying hens seemed to prefer light including UV over control light, light treatment did not appear to have a strong effect on most behaviors, except for stretching. Laying hens changed their behaviors with age and a couple of behaviors were associated with time of day.

3.1 Home pen observations

3.1.1 Scan sampling

Overall, the most frequent behaviors were foraging (25%), standing (19%) and perching and locomotion (both 13%), see figure six. None of the behavioral differences due to light treatment were found to be significant, see table two. The D65 hens had numerically slightly less occurrences of foraging and more occurrences of resting compared to the other treatments, while Jungle hens had most occurrences of perching and the least occurrences of preening.

For a couple of behaviors, age affected behavior significantly during the trial, see table four and figure six. Standing peaked around week 12, locomotion increased as the hens grew older, while resting and perching overall decreased after a rise at the start. The increased locomotion is also responsible for the effect of active behaviors (locomotion + forage) as foraging behavior remained fairly constant during the experiment. See figures seven, eight, nine and ten. Some behaviors were observed more in the morning compared to the afternoon, or vice versa. Two trends were observed (both $P = 0.072$): Laying hens performed more feeding during morning observations while dust-bathing was more frequent in the afternoon.

3.1.2 Continuous sampling

There is a large variation between occurrences of behavior, with the least frequent severe pecking (total 14) and the most frequent running (total 1073), see figure 11. Other behaviors quite commonly seen were gentle pecking, wing-shaking and sparring. When looking at active behaviors such as aggression, running and sparring it seems that D65 hens had slightly less occurrences compared to the other treatments, though not significantly different. Jungle hens were observed most often gentle pecking – almost three times more compared to Control hens – while Control hens performed most aggressive behavior. However, none of these findings were significant when tested, see table five. The only finding statistically relevant was a trend for D65 hens to stretch more compared to their Jungle and Control counterparts.

For the social behaviors scored in continuous sampling most were clearly affected by age (table five). The only exception was severe peck, which was barely observed during the whole trial. Running was one of the most observed behaviors at young age, yet decreased to be one of the least observed behaviors at week 21. A small peak in running can be observed at week 12, which corresponds with a sharp peak for wing-shake and a peak for gentle peck. Aggression gradually increased with age and also vocalizations were observed more frequently towards the end of the experiment. Sparring seemed to be slowly rising at first, but went down at week 21. Stretching behavior was not observed much, but was seen most at the age of eight weeks. The most relevant data for the development of single behaviors per light treatment are presented in figures 12 to 15.

Some behaviors were found to occur significantly more in the morning than in the afternoon (table five). On average running occurred almost twice as often during the morning observations (11.02 ±1.33) compared to the afternoon observations (6.87 ±1.04). Vocalizations were heard about three times more frequent during the morning observations (3.27 ±0.63 vs. 0.83 ±0.15). Mean occurrences of social active behavior were on average significantly higher in the morning compared to the afternoon. See table five.

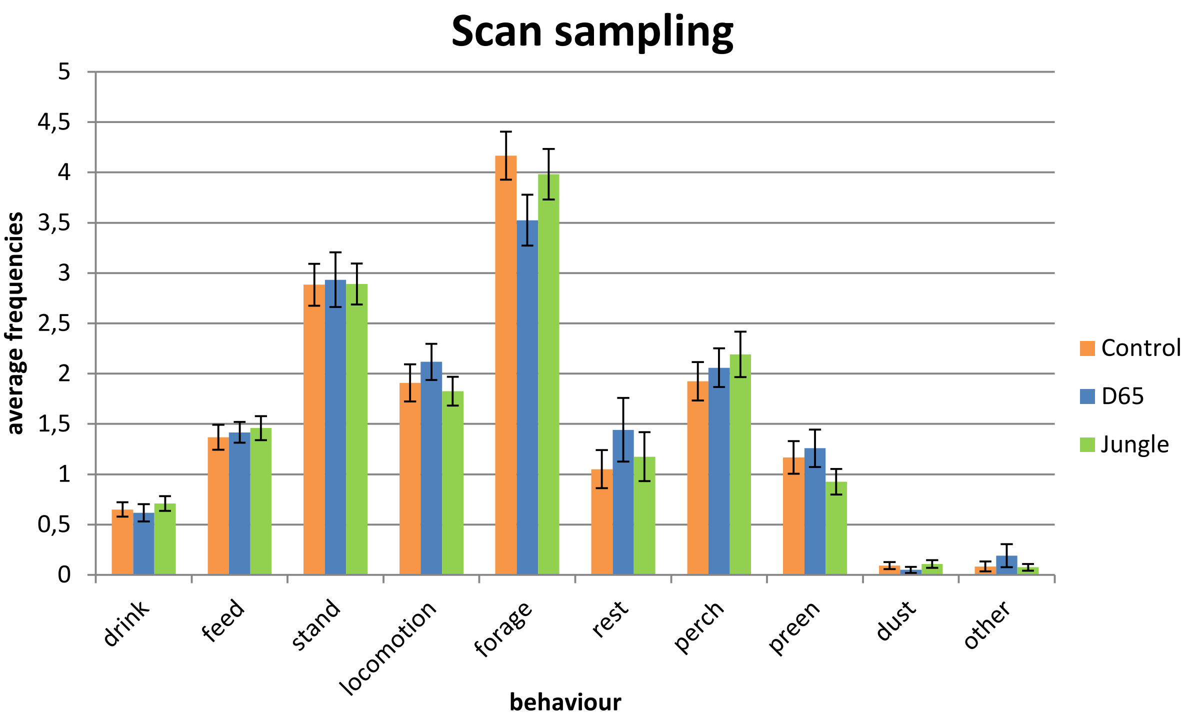

Figure 6. The average occurrence of behaviour during the home pen scan sampling observations.

Table 4. *Test results home pen observations, scan sampling*

Behaviour	Light source	Age	Time
Drink	ns	ns	ns
Feed	ns	ns	P = 0.072
Stand	ns	P = 0.000	ns
Locomotion	ns	P = 0.000	ns
Forage	ns	ns	ns
Rest	ns	P = 0.000	ns
Perch	ns	P = 0.000	ns
Preen	ns	ns	ns
Dust-bathe	ns	ns	P = 0.072
Active behaviour	ns	P = 0.002	ns
Comfort behaviour	ns	ns	ns

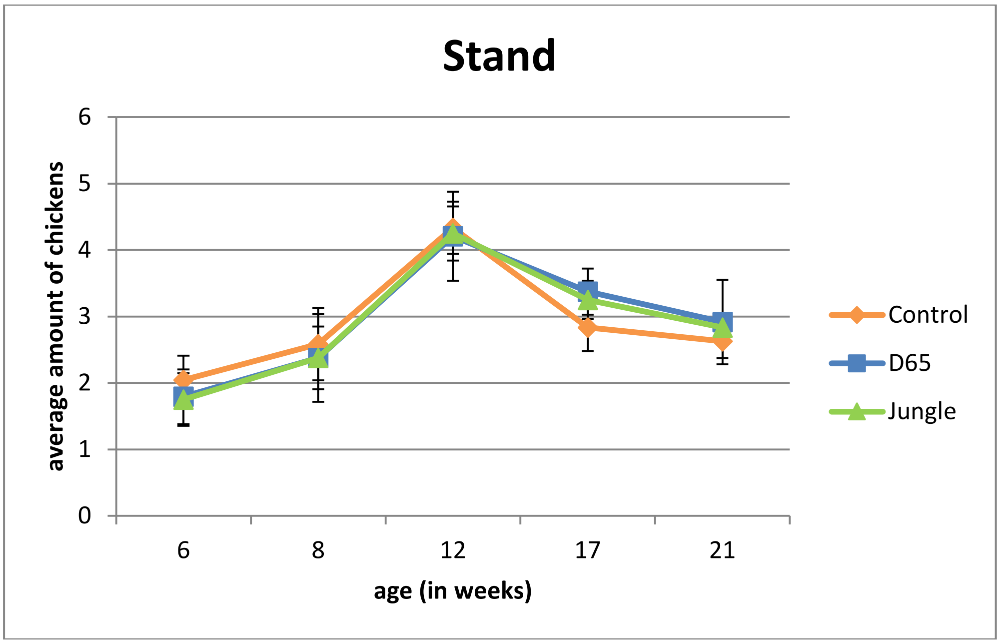

Figure 7. Development of standing behaviour for the different light treatments.

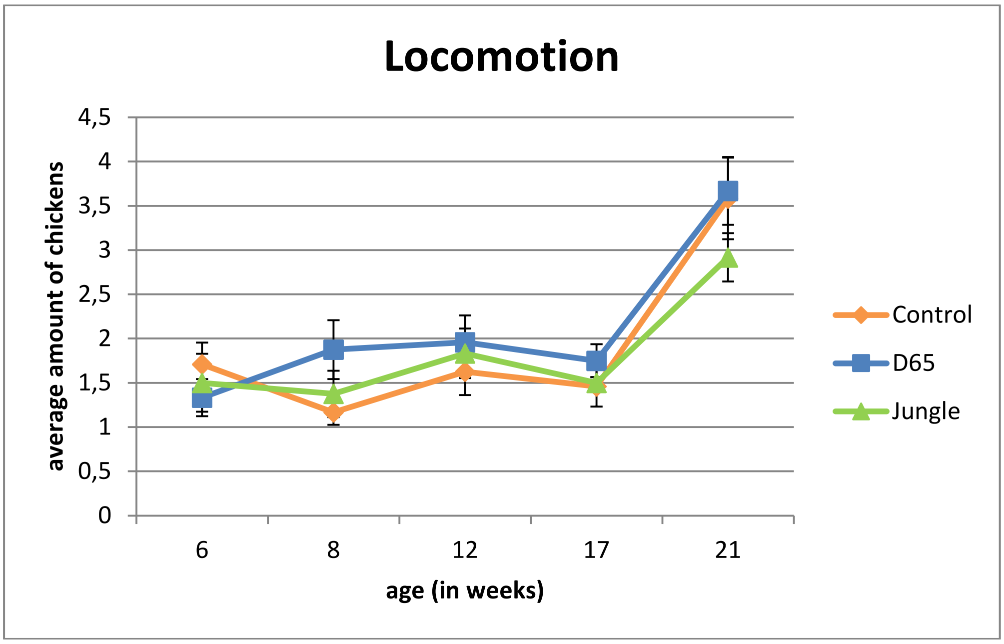

Figure 8. Development of locomotive behaviour for the different light treatments.

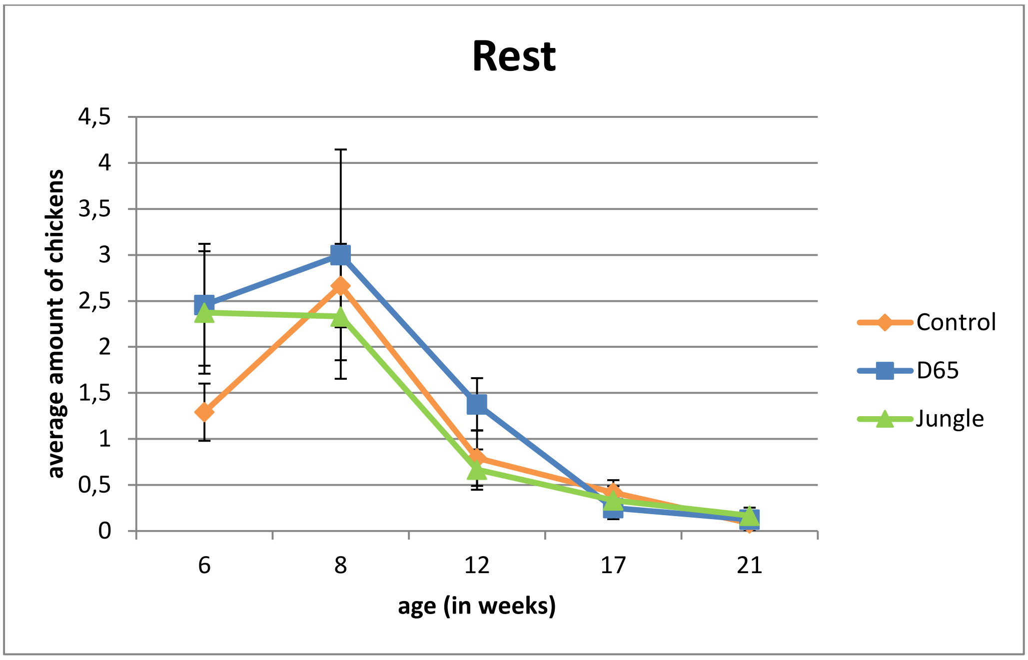

Figure 9. Development of resting behaviour for the different light treatments.

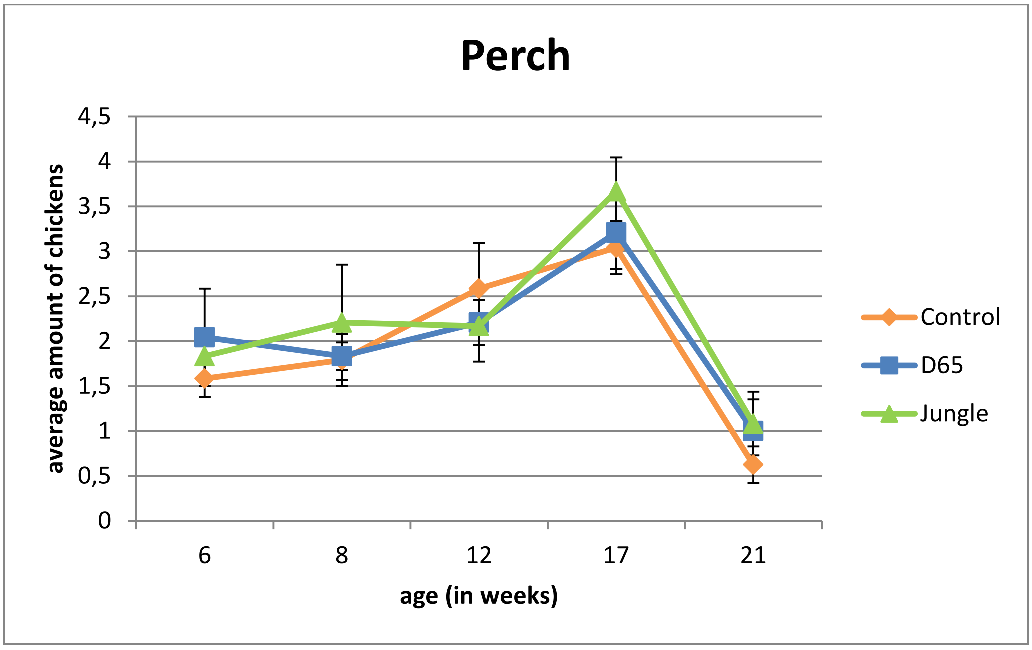

Figure 10. Development of perching behaviour for the different light treatments.

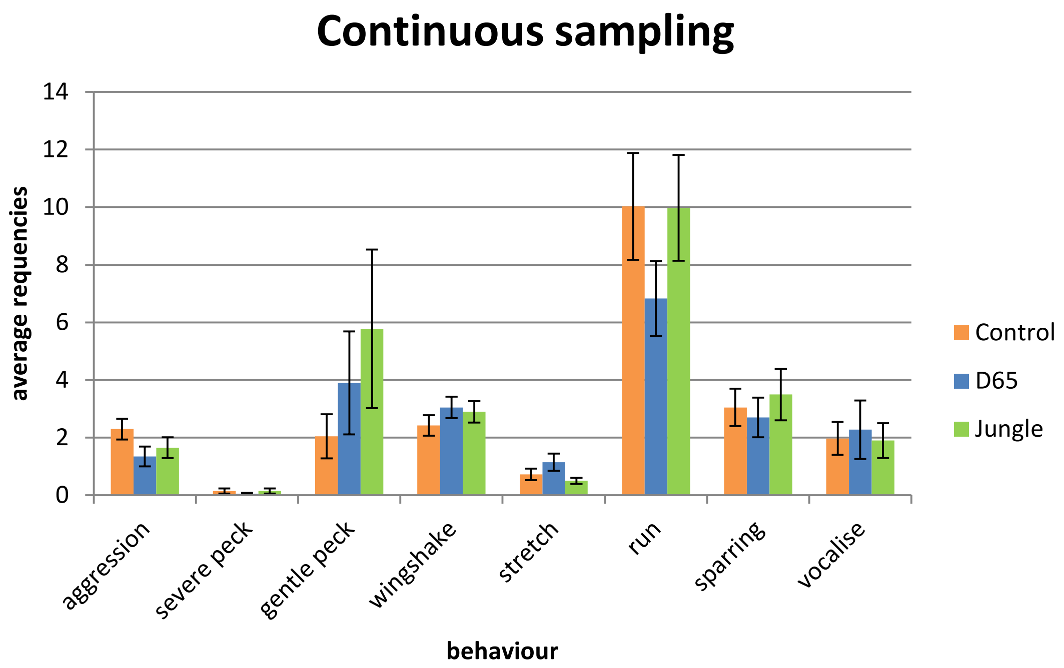

Figure 11. The average occurrence of behaviour during the home pen continuous sampling observations.

Table 5. *Test results home pen observations, continuous sampling*

Behaviour	Light source	Age	Time
Aggression	ns	P = 0.000	ns
Severe peck	ns	ns	ns
Gentle peck	ns	P = 0.037	ns
Wing-shake	ns	P = 0.000	ns
Stretch	P = 0.097	P = 0.001	ns
Run	ns	P = 0.000	P = 0.018
Sparring	ns	P = 0.000	ns
Vocalize	ns	P = 0.000	P = 0.001
Comfort behaviour	ns	P = 0.001	ns
Social active behaviour	ns	P = 0.000	P = 0.018

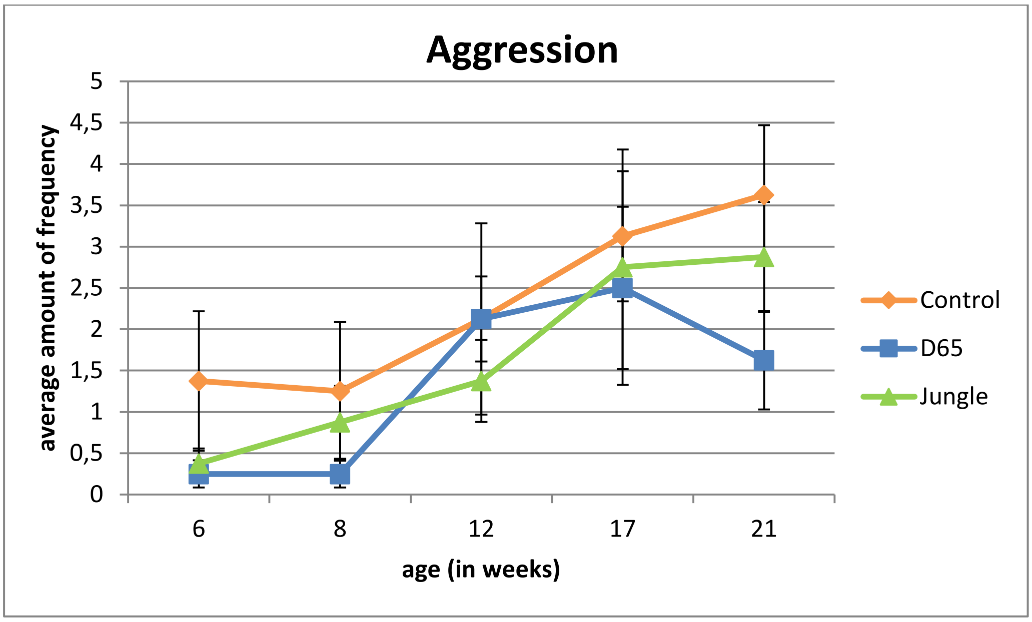

Figure 12. Development of aggressive behaviour for the different light treatments.

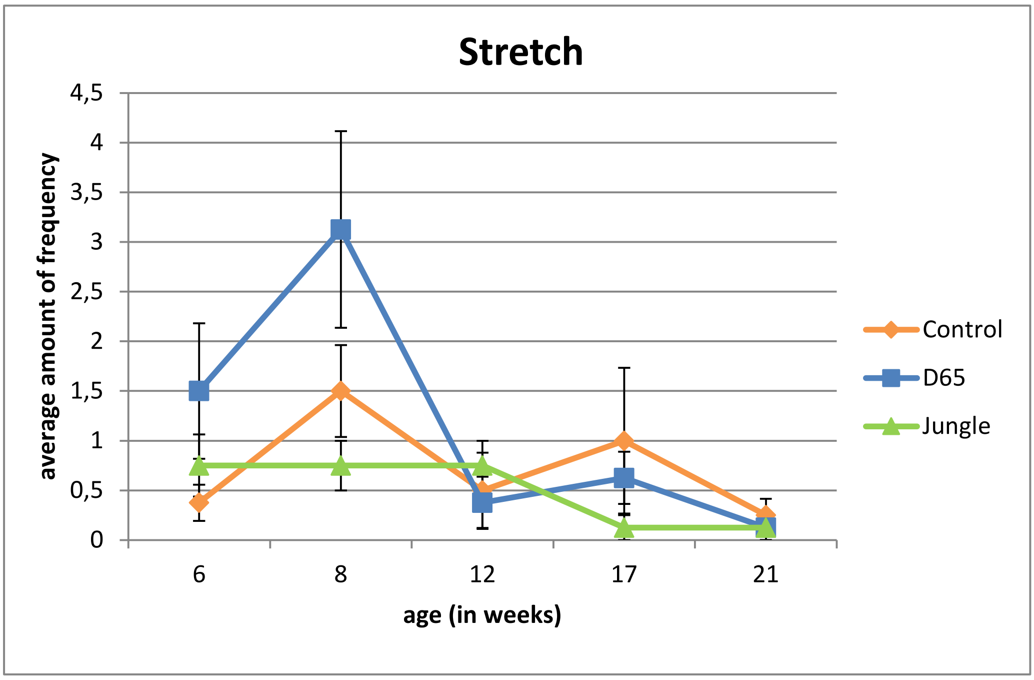

Figure 13. Development of stretching behaviour for the different light treatments.

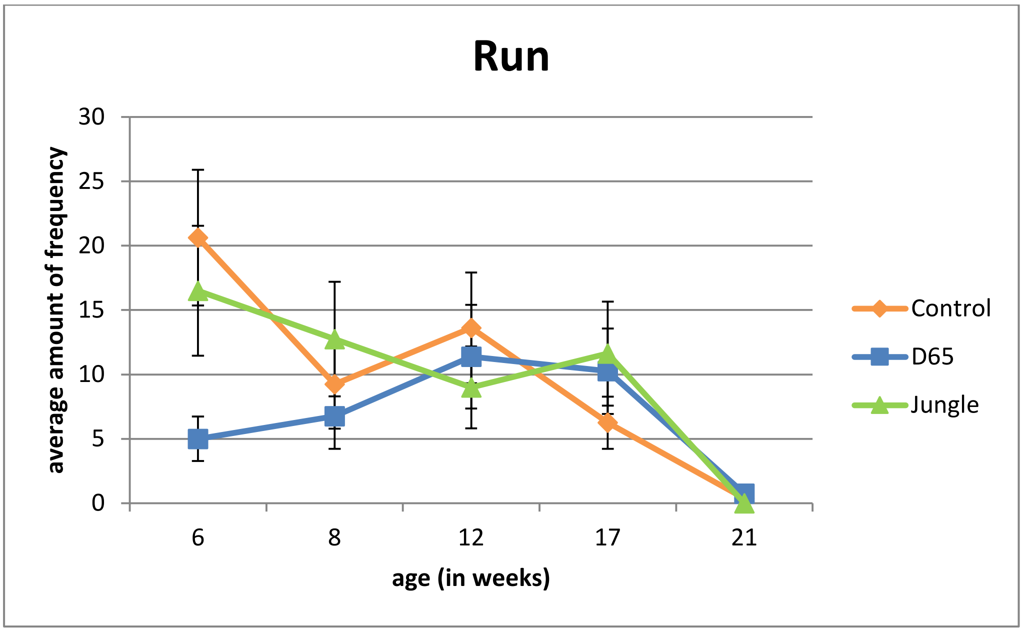

Figure 14. Development of running behaviour for the different light treatments.

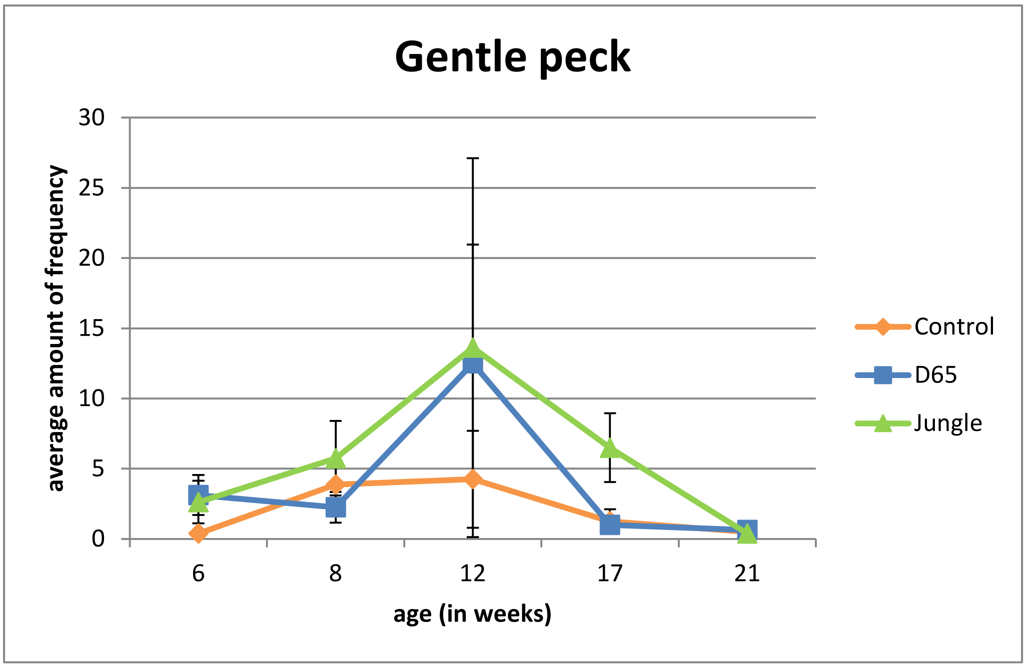

Figure 15. Development of gentle pecking behaviour for the different light treatments.

3.2 Preference tests

3.2.1 Occupancy

There was a tendency in overall preference for the different light treatments in the preference test (Glimmix; P = 0.0504, F = 3.16). LSM indicates that jungle light is preferred compared to control (P = 0.024), and a tendency for a preference for D65 compared to control (P = 0.0521). When given the choice between D65 and jungle, 52.5% of the hens spent time in D65, though this difference was not significant. See table six below for the percentages of average occupancy in the preference tests.

Table 6. *Average occupancy in preference tests (including SE) in percentages; * $P \leq 0.1$, ** $P \leq 0.05$*

Control	D65 *	Control	Jungle **	D65	Jungle
48.7 ± 2.3	51.3 ± 2.4	45.6 ± 1.9	54.3 ± 2.0	52.5 ± 2.5	47.5 ± 2.1

3.2.2 Home pen light

There was no significant effect of home light, nor interaction between light and home light. Though not significant, for both the hens raised in the light treatments of D65 and jungle there were slightly more occurrences in the treatments of their home pen light – when available. And when their home pen light was not available, hens raised in jungle light had slightly more occurrences in D65 compared to control and hens raised in D65 had slightly more occurrences in jungle compared to control. Hens raised in control light had slightly more numerical occurrences in the light they were raised in when given the novel option of D65, but this was not the case if the novel option was jungle light. When control hens were given the choice between the two new light environments of D65 and jungle light, they had slightly more occurrences in D65 light. None of these findings were found to be statistically relevant. See table seven below for the average occupancy during the preference tests in relation to home pen light.

Table 7. *Average occupancy in preference tests (including SE) in relation to home pen light in percentages*

Home light	Control	D65	Control	Jungle	D65	Jungle
Control	55.0 ± 5.5	45.0 ± 4.8	45.7 ± 2.1	54.3 ± 1.9	54.0 ± 4.5	46.0 ± 3.8
D65	45.4 ± 2.8	54.6 ± 3.0	46.9 ± 5.2	53.1 ± 4.5	55.9 ± 4.7	44.1 ± 3.5
Jungle	45.8 ± 2.8	54.2 ± 2.5	44.2 ± 1.6	55.8 ± 4.3	47.6 ± 2.9	52.4 ± 3.3

3.2.3 Behavior

Several behaviors were observed to be unequally distributed over the light treatments, see figures 16, 17 and 18. For example dust-bathing was a popular behavior in control light, when the option was available. Drinking and foraging were less popular in control light if D65 or jungle light was the alternative option. If the choice was between the two UV treatments – jungle and D65 – the differences in behavior were more subtle compared to choice including control light. Dust-bathing and preening were a bit more frequent in D65 light compared to jungle, while the other behaviors were fairly equally distributed.

As perching was not categorized as a behavior but as a location during the preference tests, perching behavior was measured by occupancy of the perch. The results of the average occupancy per area in the preference tests are shown in table eight. Average perching occurrences per light section seem not to be in line with average occurrences in the slats and litter section per treatment. Based on the descriptive data, it seems that jungle light is most favorable option for the laying hens to perch at. Least occurrences of behavior on the perch took place in the D65 light section.

Table 8. *Average occupancy in preference tests (including SE), specified for area*

Area	Control	D65	Control	Jungle	D65	Jungle
slats	47.5 ± 2.9	52.5 ± 3.4	45.4 ± 1.9	54.6 ± 2.4	53.9 ± 4.3	46.1 ± 3.4
litter	47.6 ± 3.3	52.4 ± 3.2	46.3 ± 2.7	53.7 ± 4.0	52.6 ± 3.8	47.4 ± 3.6
perch	58.1 ± 17.2	41.9 ± 9.2	43.6 ± 13.9	56.4 ± 14.1	46.2 ± 12.5	53.8 ± 10.1

3.2.4 Behavior in relation to home pen light

For some behaviors there were numerically more occurrences in the section of their home pen light in comparison to the relative occurrence for that behavior for a given light combination (table nine). For the preference tested between control and D65 this seems to be the case for hens raised in control light in regard of all behaviors. However, for laying hens raised in control light, hens have a higher statistic for jungle in the case of drinking, dust-bathing, foraging, locomotion and resting. For hens raised in D65, all behaviors are on average relatively higher in the D65 section when the choice is between control and D65 light. When the choice is between D65 and jungle only, resting is slightly lower in D65 light for hens raised in D65. For hens raised in jungle light, there is a relatively higher average occurrence of drinking, dust-bathing, foraging and locomotive behavior in the control section when the choice is between control and jungle light. The opposite was found for feeding, standing, resting and preening, which were observed relatively more frequently. In the preference test between D65 and jungle light, there seems to be a preference for the jungle section by jungle raised hens for most of the behaviors, with the exception of dust-bathing and resting.

When observing novel choices for the hens, the preference of control-raised hens and D65-raised hens, is quite evenly spread over the different behaviors.

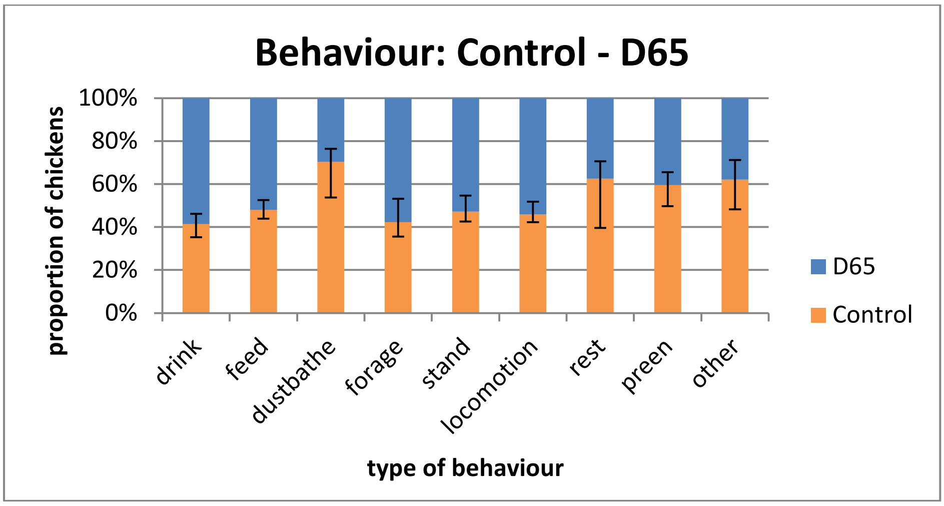

Figure 16. Frequency of behaviors observed during the preference tests between control and D65.

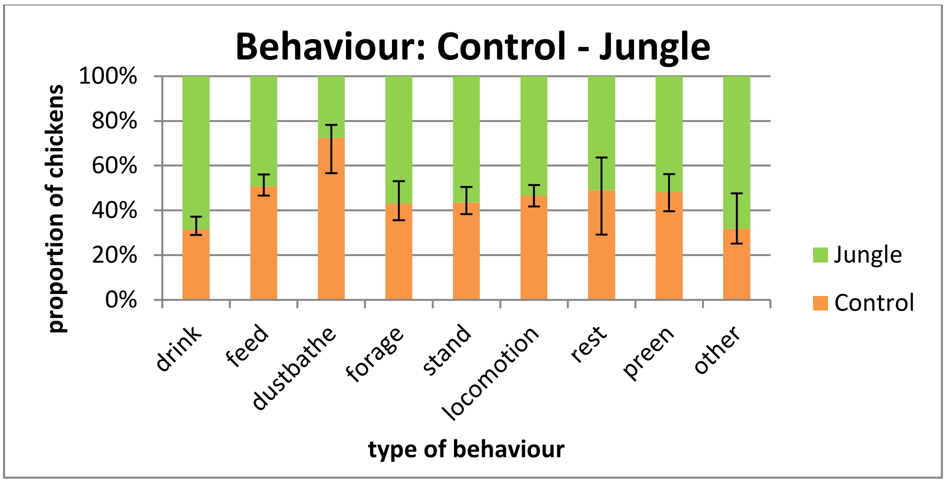

Figure 17. Frequency of behaviors observed during the preference tests between control and jungle.

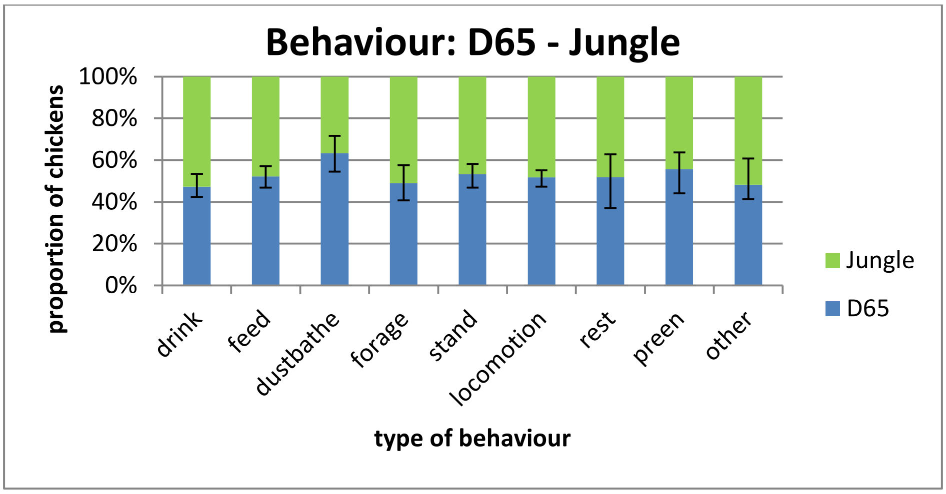

Figure 18. Frequency of behaviors observed during the preference tests between D65 and jungle.

Table 9. *Mean percentages of laying hens (including SE) performing a type of behavior per light section during the preference tests*

Homelight	Control	D65	Control	Jungle	D65	Jungle
Control						
drink	48.0 ± 14.0	52.0 ± 5.5	29.9 ± 5.6	70.1 ± 5.7	47.9 ± 11.2	52.1 ± 14.7
feed	58.3 ± 9.3	41.7 ± 6.4	52.8 ± 6.3	47.2 ± 8.3	59.0 ± 7.9	41.0 ± 2.4
dustbathe	74.8 ± 29.6	25.2 ± 15.5	66.1 ± 6.9	33.9 ± 3.6	56.3 ± 17.8	43.7 ± 14.9
forage	47.3 ± 8.3	52.7 ± 18.3	41.8 ± 15.0	58.2 ± 22.5	53.9 ± 18.4	46.1 ± 16.8
stand	52.1 ± 7.8	47.9 ± 9.4	44.7 ± 8.5	55.3 ± 11.4	54.5 ± 18.0	45.5 ± 8.3
locomotion	52.1 ± 6.5	47.9 ± 7.0	44.8 ± 7.4	55.2 ± 12.6	55.0 ± 12.7	45.0 ± 8.3
rest	72.8 ± 55.1	27.2 ± 10.5	43.2 ± 12.4	56.8 ± 21.3	49.1 ± 15.0	50.9 ± 12.7
preen	64.9 ± 23.2	35.1 ± 7.3	64.9 ± 23.2	35.1 ± 7.3	50.7 ± 9.0	49.3 ± 19.7
other	85.1 ± 18.8	14.9 ± 6.1	85.1 ± 18.8	14.9 ± 6.1	41.8 ± 13.9	58.2 ± 31.7
D65						
drink	39.7 ± 8.7	60.3 ± 3.5	30.1 ± 3.1	69.9 ± 10.9	52.5 ± 5.3	47.5 ± 7.1
feed	45.6 ± 4.6	54.4 ± 3.4	52.5 ± 6.0	47.5 ± 9.7	54.5 ± 15.8	45.5 ± 10.1
dustbathe	60.8 ± 15.6	39.2 ± 13.1	80.2 ± 25.0	19.8 ± 7.7	65.9 ± 21.0	34.1 ± 6.8
forage	37.5 ± 11.6	62.5 ± 23.9	41.6 ± 15.3	58.4 ± 20.4	50.2 ± 5.7	49.8 ± 10.6
stand	44.5 ± 6.2	55.5 ± 11.3	44.3 ± 12.7	55.7 ± 16.0	59.9 ± 7.5	40.1 ± 5.8
locomotion	44.9 ± 5.9	55.1 ± 4.2	46.5 ± 11.3	53.5 ± 6.8	52.5 ± 5.2	47.5 ± 7.5
rest	47.6 ± 13.0	52.4 ± 12.0	59.9 ± 31.6	40.1 ± 23.1	50.3 ± 33.1	49.7 ± 15.6
preen	58.8 ± 11.1	41.2 ± 13.4	51.3 ± 12.2	48.7 ± 19.2	60.5 ± 29.6	39.5 ± 11.2
other	41.9 ± 17.6	58.1 ± 19.5	32.7 ± 13.8	67.3 ± 36.0	69.4 ± 6.3	30.6 ± 15.6
Jungle						
drink	36.2 ± 7.6	63.8 ± 11.3	35.4 ± 5.1	64.6 ± 10.9	44.5 ± 6.7	55.5 ± 8.2
feed	40.1 ± 4.1	59.9 ± 11.2	46.0 ± 10.2	54.0 ± 12.7	44.4 ± 4.9	55.6 ± 7.8
dustbathe	72.4 ± 27.0	27.6 ± 6.3	73.7 ± 68.1	26.3 ± 13.7	66.2 ± 9.1	33.8 ± 21.3
forage	40.2 ± 14.2	59.8 ± 21.1	46.1 ± 11.6	53.9 ± 14.0	42.6 ± 15.5	57.4 ± 20.1
stand	46.0 ± 11.2	54.0 ± 16.2	41.2 ± 7.7	58.8 ± 11.5	48.4 ± 9.6	51.6 ± 9.4
locomotion	42.0 ± 7.0	58.0 ± 12.4	48.5 ± 7.3	51.5 ± 6.5	48.2 ± 4.5	51.8 ± 3.5
rest	60.2 ± 26.8	39.8 ± 15.5	36.6 ± 15.5	63.4 ± 23.4	56.7 ± 16.6	43.4 ± 23.0
preen	54.9 ± 19.0	45.1 ± 11.9	45.3 ± 19.5	54.7 ± 3.9	54.2 ± 13.1	45.6 ± 8.4
other	59.1 ± 31.5	40.9 ± 14.4	30.1 ± 12.7	69.9 ± 22.6	39.2 ± 13.6	60.8 ± 16.8
Overall						
drink	41.4 ± 6.0	58.6 ± 4.8	31.5 ± 2.6	68.5 ± 5.6	48.2 ± 4.9	51.8 ± 6.1
feed	47.9 ± 4.0	52.1 ± 4.7	50.5 ± 4.4	49.5 ± 5.4	52.2 ± 5.3	47.8 ± 5.0
dustbathe	70.3 ± 16.5	29.7 ± 6.0	72.6 ± 15.7	27.4 ± 5.6	62.4 ± 8.8	37.6 ± 8.4
forage	42.2 ± 6.7	57.8 ± 10.9	43.3 ± 7.4	56.7 ± 9.9	49.4 ± 8.3	50.6 ± 8.4
stand	47.2 ± 4.7	52.8 ± 7.4	43.5 ± 5.4	56.5 ± 7.0	54.1 ± 6.4	45.9 ± 4.8
locomotion	45.9 ± 3.6	54.1 ± 5.8	46.6 ± 4.9	53.4 ± 4.7	51.8 ± 4.4	48.2 ± 3.4
rest	62.6 ± 23.0	37.4 ± 8.0	50.4 ± 18.8	49.6 ± 13.8	50.7 ± 14.5	49.3 ± 10.6
preen	59.5 ± 9.8	40.5 ± 5.9	49.2 ± 8.2	50.8 ± 7.8	55.4 ± 11.5	44.6 ± 7.9
other	62.1 ± 13.8	37.9 ± 9.1	32.1 ± 6.6	67.9 ± 16.1	48.9 ± 7.1	51.1 ± 12.7

Control-raised hens spent relatively more time feeding, foraging, standing and locomotive behavior in the D65 section, versus relatively more time drinking, dust-bathing, resting and preening in the jungle section. Hens raised in D65 show relatively more feeding, dust-bathing, standing, resting and preening in the control section against relatively more drinking, foraging and locomotion in the jungle section. Jungle-raised hens showed a higher frequency for almost all behaviors in the D65 section, when the choice was between control and D65, with the exception of dust-bathing.

3.2.5 Time

The descriptive data on time show a clear change at the end of the day, when the average scores for control light are around six and a half hens, against over eight hens on average observed in both D65 and jungle.

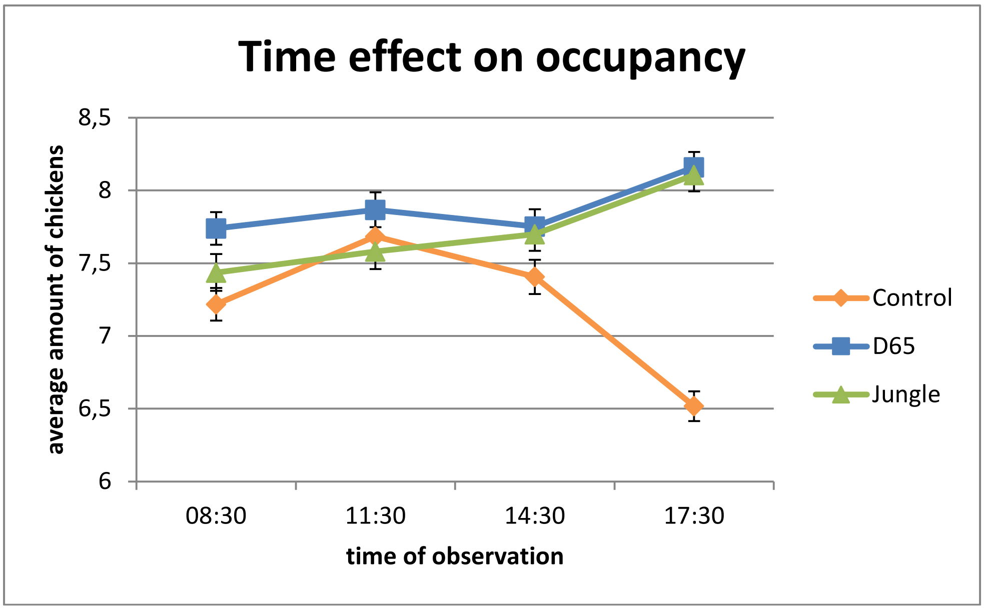

Figure 19. Time effect on average overall occupancy in the different light sections during the preference tests.

4 Discussion

The results of the home pen observations describe that the behavior of the laying hens is affected by age and time of day, but not by light treatment. The results of the preference tests show a preference for UV-enriched light sources when the option is available.

There were no significant differences in behavior during home pen observations between light treatments, besides a trend of stretching behavior to occur more frequently in D65 hens. This finding is not in line with previous research which has indicated that light spectrum can affect behavior in chickens. However, in those studies the spectral differences were more obvious, as red light was compared with blue and green light (Prayitno *et al*., 1997a; Prayitno *et al*., 1997b; Sultana *et al*., 2013). One explanation could be that the differences in spectral composition in our experiments were too subtle for the laying hens to result in a significant effect. Yet, other studies comparing light from different light sources – all emitting light of similar color – still found differences for a couple of behaviors (Widowski *et al*., 1992; Kristensen *et al*., 2007). Perhaps it could be that in our study treatment light was not offered early enough in rearing.

Another explanation might be that the differences in the shorter range of the light spectrum (the ultraviolet range) may have less or no effect on behavior compared to those differences in the longer range (red light). Similarly Maddocks et al. (2001), who showed significantly higher basal plasma corticosterone concentrations in UV-deficient environments, found in the same experiment no significant differences in behavior between the UV-deficient and full spectrum groups of chickens – though few trends were observed. Our finding of a trend for stretching behavior being more frequent in D65 light is not the first study to point out that spectrum might affect stretching behavior in chickens. Sultana et al. (2013) showed broilers stretching behavior to be affected by spectral composition as well.

Age had a significant effect on a couple of the behaviors and specifically on most of the social behaviors. Age affecting bird behavior is in line with findings from multiple studies (Savory & Maros, 1993; Kjaer & Vestergaard 1999; Sultana *et al*., 2013). Gentle peck, wing-shaking and standing behaviors all had a peak around week 12 (see appendix). These changes in behavior might be due to the juvenile molt laying hens commonly go through around 12 weeks of age.

Feeding, running, vocalizations and social active behaviors were observed more during the mornings, while dust-bathing was observed most frequent during the afternoon. Other studies in the field of behavior in relation to light spectrum do not confirm this finding, for example Sultana et al. (2013) did not find an effect from time of day on the behavior of broiler chickens. One explanation for this difference in findings could be that, with the exception of feeding, none of the behaviors which scored a significant difference or trend for time of day, were part of the research as performed in the study by Sultana et al. (2013). Another explanation could be that the findings for time of day effect in our study is related to the dark period overnight, while in the study of Sultana et al. (2013) broilers were raised in continuous lighting. A behavioral study by Savory and Maros (1993) did find effect from time of day on most behaviors, but categorized time of day in relation to feeding moments, which was – in contrast to our study – restricted in their research.

When hens were allowed to choose between light environments, there was a preference for jungle light compared to control and a tendency of preference for D65 compared to control. In both cases the result shows a preference for a UV-enriched light environment. This favored choice might suggest that UV enrichment could lead to improved welfare in laying hens. The idea that UV light might be important to chickens is in line with previous studies suggesting a relation between negative welfare and UV-deficiency (Jones *et al*., 2001; Maddocks *et al*., 2001). However as this study did not convincingly show a strong, significant preference for UV enriched environments either, further research would be recommended to confirm or disprove the idea of relation between UV-deficiency and negative welfare.

There was no significant difference in preference between D65 and jungle treatment. This could indicate that while UV-enrichment is important to hens, the spectral composition of jungle light does not have an added value over normal lighting. Contradictory, the preference for jungle light compared to control was significant, while D65 compared to control was a trend only, which gives reason to believe that the spectral composition of jungle light (more green) does have an added value to hens compared to D65 (more blue). In the study of Prayitno et al. (1997a) preference was found for blue light for chicks raised in green, red and white light, while chicks raised in blue light had developed preference for a green light environment. Both of these studies show a specific interest for blue and green illumination, yet do not confirm each other's findings. Further research into the role of spectral composition for the wellbeing of chickens is therefore recommended.

According to our results, home pen light had no significant effect on the laying hens' preference for light treatment. In contrast, Prayitno et al. (1997a) found preference for colored light to be affected by the color that meat chicks were reared at. One possible explanation for this difference in outcome could be that the chicks in the study of Prayitno et al. (1997a) were already reared in treatment light from week two, whereas the chicks in our study were started with light treatments from week five.

Abnormal behaviors were rarely observed during this study. It might be that the light spectra of the lights used in the experiment had a positive effect, yet the finding that no differences in occurrence of abnormal behavior were found between treatments does not strengthen this line of thinking. One explanation could be that the low average light intensity prevented from feather pecking, as the average illumination during home pen observations was measured to be below ten lux. Also age is known to affect feather pecking behaviors and our study took mainly place during young age of the laying hens. Another explanation could be that our home pens were more spacious compared to the commercial standard. It is not unlikely to imagine that a combination of factors might have prevented from abnormal behaviors, regarding the multi-factorial nature of aggressive feather pecking.

4.1 Strengths

One of the strengths of our research was the use of custom built lights which were actual representations of the light environments to the ancestor of the laying hen, the red jungle fowl. Also including the commercial standard makes the study valuable for connecting it to actual practices in the industry. Another strength of this study could be the combination of two related experiments. The home pen light studies and the preference studies are two independent measures, yet the combination gives added value as it gives insight into the interaction between home pen light and preference.

Between-observer reliability during the home pen observations was an asset for this study. There were only two observers which had assessed and improved their between-observer reliability during the pilot beforehand, after which the ethogram was fine-tuned as well by adding several behaviors to the protocol. Furthermore during the pilot assessments were made for different group sizes and observation intervals in relation to scoring percentages, before deciding on the final form of the sampling method. A strength to the preference tests was the use of cameras, which allowed observations without distracting hens from their normal behavioral patterns.

To the authors' knowledge, a similar study has not been performed with laying hens before the date of publishing this degree project. The approach of combining behavioral studies during rearing under different spectral compositions with spectral preference studies has been seen in broilers before (Prayitno *et al.*, 1997a; Kristensen *et al.*, 2006), yet is novel when it comes to the research in laying hens. Furthermore analyzing whether time of day could possible affect spectral preference has – to the authors' knowledge – not been researched in any kind of poultry.

4.2 Limitations

The first weeks of rearing in this experiment did not happen under the designated home pen lights. Besides the shorter amount of weeks of exposure to the treatment factor, exposure during the sensitive period might have had stronger effects as well.

Jones and Waddington (1992) argued chicks to be still susceptible to learning experiences until day nine, while our treatment was started in week five. During the later stage of rearing all UV lights broke and birds in the UV treatments (D65 and Jungle) were not exposed to UV lighting for an unknown period of time. Therefore the results may have shown less powerful differences between groups as might have been the case if treatments had been started earlier during rearing and if UV lights in the UV treatments would have functioned well throughout the whole experiment.

A challenge for the light environment during our experiments was to have an equal light distribution throughout the pens (see appendix). The D65 and jungle light sources were very different from shape compared to the control light sources. Additionally, the UV lights in the D65 and jungle treatment had to be attached separate from the main source and the electricity sockets did not allow to locate them close together. On the ground level the effect in light distribution was probably less noticeable, but when the birds would be higher up on the perch it might be imaginable that the differences were more apparent.

Even though it was attempted to reach the same light intensity in all the home pens, the average intensity varied between 7,0, 11,0 and 8,0 lux for control, D65 and jungle treatment respectively. In the preference pens the variation was smaller, yet the average intensity varied between the different sections. On average this resulted the control sections to be illuminated by 6.52 (± 0.17) lux, D65 sections by 7.18 (±0.34) lux and the jungle sections by 7.52 (± 0.14) lux. These differences in light intensity might have affected the outcomes of both the behavioral observations as well as the hens' preference.

Furthermore perceived light intensity for chickens is known to be very different from light intensity measured by a photometer and expressed in lux (Nuboer *et al*., 1992). As wavelength can change the apparent intensity of the light to birds (Prayitno & Phillips, 1997), it can be challenging to distinguish the direct effects of wavelength from the effects of intensity (Manser, 1996). Prayitno & Phillips (1997) showed through behavioral testing that chickens identify lights of equal intensity, when blue light is three times as bright as red light. It should be kept in mind that our study did not take these differences of chicken light perception into consideration and that results found, could partly be due to differences in perceived intensity as well.

5 Conclusions

Laying hens still have a preference for light spectra that imitate the light environment of their ancestors compared to light conditions in modern poultry housing. Behavior was not significantly affected by light spectra used in this study and the spectrum during rearing did not alter preference for light environment and behavior later in life. Based on preference, the optimal light spectrum to laying hens seems to be similar in spectral composition to the chicken's ancestral light environment.

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