Spatial resolution threshold in layers and red jungle fowls – Are there differences due to light intensity or light spectra?

Tröskelvärden i spatial upplösningsförmåga hos värphöns och röda djungelhöns – Finns det skillnader som beror på ljusintensitet eller ljusspektra?

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Abstract

The domestic fowl (*Gallus gallus domesticus*) originates from the forests in South-east Asia. It may be correct to assume that the visual system of the domestic fowl is still adapted to light that is filtered through the green forest canopy. In modern poultry production, however, light is provided via artificial luminaires and in low intensities to control feather pecking and cannibalism. Compared with production and reproduction, the effect of light intensity on visual abilities is not researched as much. The aim with this study was to compare spatial resolution threshold between layers and its ancestor the red jungle fowl, between different light spectra and light intensities.

Chickens were held in one of three light treatments during part of the rearing, and during training and testing. One light treatment represented the light in the natural habitat of red jungle fowl, called the jungle light. Another light treatment was the standardized illuminant D65 which represents average daylight. The control light did not have UV-light, which can be discriminated by birds, included as the two other light treatments had. Twelve layers and twelve jungle fowls were trained to discriminate between a square-wave grating and a homogenous grey picture inside a Skinnerbox and tested in four different light intensities. Each bird was tested with increasing spatial frequency of the grating, and when discrimination failed, the spatial resolution threshold of that chicken was said to have been reached.

A significant difference in spatial resolution threshold was found between light treatments for red jungle fowls. The group with D65-light had a mean threshold of 5.023 cycles per degree of visual angle (c/deg) compared with 3.794 c/deg in the jungle light group (p-value < 0.05). Significant differences were also found between the breeds in the lowest light intensity in the jungle light and control light treatment. In the control light group, the mean spatial resolutions of the layers was 4.095 c/deg and 2.783 c/deg among the red jungle fowl (p-value < 0.05). In the jungle light group, the mean spatial resolution of the layers was 4.017 c/deg and 2.373 c/deg among the red jungle fowl (p-value < 0.05). A significant difference in spatial resolution threshold between the breeds in the lowest light intensity may be seen as an evidence that layers have adapted to the dim light conditions which is common in commercial layer facilities.

To further deepen the understanding of how spatial resolution threshold is affected by light spectra, testing in one colour of light at a time would be interesting, as well as measuring production and welfare parameters in a larger scale behavioural study with the jungle light.

Keywords: spatial resolution threshold, layer, red jungle fowls, visual acuity

Sammanfattning

Den domesticerade hönan (*Gallus gallus domesticus*) härstammar från skogarna i sydvästra Asien. Man kan anta att de visuella förmågorna hos domesticerade hönsfåglar fortfarande är anpassade till den sortens ljus som filtreras genom den gröna växtmassan. I modern äggproduktion tillförs ljus istället via artificiell väg och i låga ljusintensiteter för att kontrollera fjäderplockning och kannibalism. Jämfört med produktionsegenskaper och reproduktion har det inte forskats lika mycket på visuella förmågor hos höns. Syftet med denna studie var att jämföra den spatiala upplösningsförmågan mellan värphöns och dess anfader röda djungelhöns, mellan olika ljusspektrum och mellan olika ljusintensiteter.

Kycklingarna i studien hölls i en av tre olika ljusmiljöer under delar av uppväxten, och under träning och tester. Ett av experimentljusen representerade det ljus som finns i de röda djungelhönsens naturliga habitat, kallat djungelljuset, och ett annat experimentljus var det standardiserade D65-ljuset som ska representera naturligt dagsljus. Kontrollljuset innehöll inte UV-ljus, som fåglar kan uppfatta, till skillnad från de två andra experimentljusen. Tolv värphönshybrider och tolv röda djungelhöns blev tränade att särskilja mellan ett fyrkantsvågsgitter och en homogen grå bild i fyra olika ljusintensiteter i en Skinnerbox. Varje fågel testades genom att öka den spatiala frekvensen av gitter och när de inte kunde särskilja mellan bilderna sades det att kycklingens högsta spatiala upplösningsförmåga hade uppnåtts.

En signifikant skillnad i spatial upplösningsförmåga fanns mellan grupperna av röda djungelhöns. Gruppen med D65-ljus hade ett medelvärde på sitt tröskelvärde på 5.023 cykler per visuell grad (c/deg), jämfört med 3.794 c/deg hos gruppen med djungelljus (p-värde < 0.05). Signifikanta resultat erhölls i jämförelsen mellan raserna i den lägsta ljusintensiteten i djungelljuset och kontrollljuset. I kontrolljusgruppen hade värphönsen ett medelvärde för spatial upplösningsförmåga på 4.095 c/deg och de röda djungelhönsen hade ett medelvärde på 2.783 c/deg (p-värde < 0.05). I djungelljusgruppen hade värphönsen ett medelvärde för spatial upplösningsförmåga på 4.017 c/deg och de röda djungelhönsen hade ett medelvärde på 2.373 c/deg (p- värde < 0.05). Ifall fler kycklingar hade inkluderats i studien hade de signifikanta skillnaderna mellan raserna i den lägsta ljusintensiteten kunnat tolkas som ett bevis för att värphöns har anpassat sig till de låga ljusintensiteterna som är vanligt förekommande inom äggproduktionen.

För att ytterligare lära sig mer om hur den spatiala upplösningsförmågan påverkas av olika ljusspektrum skulle man kunna testa den i olika separata färger, såväl som att mäta produktions- och välfärdsparametrar i en större beteendestudie i djungelljuset.

Nyckelord: spatial upplösningsförmåga, värphöns, röda djungelhöns, synskärpa

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- RJF: red jungle fowl
- ERG: electroretinograms
- VS: violet sensitive
- UVS: ultra violet sensitive
- c/deg: cycles per degree (of visual angle)
- cd/m^2: candela per m^2

1 Introduction

The domestic fowl (*Gallus gallus domesticus*) originates from the forests in South-east Asia and their visual system have therefore evolved in those distinctive light environments. Collias and Collias (1967), in their field study in northeast India, observed most jungle fowls in areas with varying heights of trees, and tall herbs and grass on the ground spaciously distributed so the fowls could walk easily in the vegetation. The light from the sun, the sky and clouds is filtered through the green forest canopy, both colour and irradiance is affected by the origin of the light spectra and by the density and seasonal changes of vegetation and day length (Endler, 1993). It may be correct to assume that the visual system of the domestic fowl is still best adapted to that type of light. In modern poultry production, however, light is provided via artificial luminaires (e.g. fluorescent, incandescent) and to a very little extent by daylight through windows (Prescott *et al.*, 2003).

It is common to keep the luminance level in poultry houses at a fairly low level, 5-10 lux, to control feather pecking and cannibalism. These behaviours occur more frequently at higher luminance levels (Prescott *et al.*, 2003). It is thought that other social behaviours are affected by dim light and therefore also impair the welfare of the fowls (Manser, 1996; Prescott *et al.*, 2003; Gover *et al.*, 2009). Keeping the light levels low in a preventative way can be problematic in aviary systems since the layers must be able to navigate securely between different heights and interior without injuring themselves. Damage to the bones can be common in these kinds of systems (Prescott *et al.*, 2003). The illumination level in bright sunshine is 100.000 lux, an overcast day 1000 lux and at twilight 10 lux approximately, which differs much from what is common practise in poultry production (Widowski, 2010). In Sweden, however, it is more common with higher luminance levels such as 20 lux and the birds must have access to daylight according to the legislation of the Swedish Board of Agriculture[1].

1. Alexandra Jeremiasson, Swedish Eggs, personal communication.

The visual system of birds is similar to that of mammals but differs in some important aspects. The spectral sensitivity of birds differs so that they have three types of photoreceptors (specialized cells in the retina which is sensitive of to certain wavelengths of light) rods, cones and double cones, while mammals only have rods and cones. They also have four photo pigments which gives them a tetrachromatic vision (Prescott *et al.,* 2003; Hart & Hunt, 2007). This combined means that birds can see a broader range of wavelengths, i.e. more colours, including ultraviolet (UV) light. Because the common unit to measure illuminance, lux, is weighted to the spectral sensitivity of humans, problems arise when we talk about poultry's perception of brightness from different light sources since their spectral sensitivity is remarkably different. This makes it impossible to compare a particular light environment between humans and fowls (Prescott *et al.,* 2003).

It has been well researched how lighting regimes affect the production and reproduction of poultry, but how the lighting regime affects the visual abilities, which is important for the birds feeding behaviour and social recognition of conspecifics, is less explored (Prescott *et al.*, 2003). This thesis is a part of a larger project that aims to find the optimal light conditions for laying hens that suit their visual system. The light conditions should give the hens the ability to search for food, recognize their peers and move around in the housing with precision. At the same time, the light conditions should give the hens a secure light environment that minimize stress and the development of problematic behaviours. This thesis will focus specifically on investigating how housing the birds in different light spectra will affect their visual abilities by examining the spatial resolution threshold among white layers and red jungle fowl (RJF) with behavioural tests.

With the aim as a background, here follows some more specialized questions I would like to answer with this thesis:

- Will the spatial resolution threshold differ between the RJF and the white layers in different light intensities since the layers have been bred and selected for egg production in stables with low light intensities for decades?
- Will the spatial resolution threshold change for the chickens if UV-light spectrum is included in the light sources?
- Will the light spectrum that simulates the light conditions found in the lower vegetation in Southeast Asia affect spatial resolution threshold of the chickens?

2 Literature review

2.1 The Red jungle fowl and the domestic chicken

2.1.1 Origin, domestication and selection

There are two hypotheses about the origin of the domestic chicken, one saying they have a monophyletic origin from the RJF only and the other saying the chicken have multiple origin from more Gallus subspecies than just the RJF (Liu et al., 2006), which are the grey jungle-fowl (*Gallus sonneratii*), green junglefowl (*Gallus varius*) and Ceylon junglefowl (*Gallus lafayetii*) (Al-Nasser *et al.*, 2007). It has been revealed that studies suggesting the first theory might come from a small sampling of birds, both domestic chickens and RJF. Research from Liu et al. (2006) and Kanginakudru et al. (2008) supports the last theory of the domestic chicken originating from several subspecies of a wild ancestor and domesticated independently in different locations in Southeast Asia. Archaeological findings have found signs of two regions of the domestication of chickens which further supports the theory of multiple domestication locations. The oldest site was found in northern China and dates from 6000 BC, the other site is in the Indus valley and dates from 2500 BC (Tixier-Boichard *et al.*, 2011).

When a population of a species is domesticated and bred in captivity, it has been habituated to human presence. It is unknown how the domestication processes of animals started but it is plausible that fearfulness of humans was a crucial part to get rid of in the beginning (Andersson *et al.*, 2001; Al-Nasser *et al.*, 2007; Agnvall *et al.*, 2012). The breeding of layers has aimed for a higher efficiency where the layers reach their maximum capacity earlier and stay at that level for a longer period of time. According to data from the Swedish egg production year 2015, a layer currently produces approximately 380 eggs until she is slaughtered between 72 and 95

weeks of age (Svenska ägg, 2015) to compare with 10 to 15 eggs per year among RJF (Al-Nasser *et al.*, 2007).

2.1.2 Difference in behaviour

The RJF prefers to have scrubland, groves, field edges and forests typical for the area in Southeast Asia (Collias & Collias, 1967). When an animal is kept close to humans in an environment which is unlike its natural habitat, behavioural and physiological changes occur. Due to this, commercial chickens and the RJF exhibit some differences, but they still share some similarities. Social behaviour, like aggressiveness and courtship, is still expressed in almost the same way in both species (Al-Nasser *et al.,* 2007).

The RJF is more fearful than the chicken, perching in trees at night to avoid predators, but they are also more explorative and investigate their environment (Andersson *et al.*, 2001; Agnvall *et al.*, 2012). Wild animals spend more time and energy on foraging than domesticated animals whose food is provided by humans. This behaviour is thought to be beneficial for wild animals since it can gain information about possible feed sources and amounts. Exploration is an energy costing behaviour which may also affect the performer in a negative way, e.g. delayed reproduction, and it is likely that these types of behaviours have been selected against during the domestication (Andersson *et al.*, 2001; Lindqvist & Jensen, 2009; Agnvall *et al.*, 2012). Domesticated chickens in a behavioural study by Lindqvist & Jensen (2009) have shown to work less hard to access feed than RJF. They theorized that domesticated chickens spend less effort on foraging since feed is accessible ad lib in commercial production.

2.1.3 Difference in visual abilities

The selection towards higher egg production and faster growth in domestic chicken might have influenced other traits or features of the sensory systems not intended for. Measurements of eye dimensions and pupil diameter at seven different light intensities in both white leghorn and RJF by Roth and Lind (2013) have shown that the increased body size in white leghorn have been followed by a proportional increased eye size, but not an increased pupil diameter. This caused a lower measured optical sensitivity in low light conditions among the white leghorns. Another visual ability among chickens which seems to have been affected by artificial selection and breeding is temporal resolution, i.e. the ability to detect fast moving objects. A study by Lisney et al. (2011) determined the highest flicker fusion frequency (FFF) at which the old Swedish game breed “Gammalsvensk dvärghöna” perceived a flick-

ering light as continuous to be 87 Hz at 1375 candela per m^2 (the SI-unit for luminance, cd/m^2) on average in a behavioural test. Gammalsvensk dvärghöna is behaviourally and morphologically similar to the RJF and it has not been bred as intensely as domestic chickens used in commercial poultry production. It should therefore still have similarities with its ancestor which the commercial chicken does not have (Lisney *et al.*, 2011). Compared with obtained critical flicker fusion frequencies (the highest frequency at any light intensity at which someone can resolve flicker, CFF) in other behavioural studies made with commercial breeds, 71.5 Hz at 1000 cd/m^2 (Jarvis *et al*., 2002) and 73.9 Hz at 800 cd/m^2 (Rubene *et al.*, 2010), Gammalsvensk dvärghöna have a relatively high CFF, i.e. can detect a flickering light while a commercial breed still perceives it as continuous. However, no definitive conclusions can be drawn until the CFF in different breeds are measured and compared in a single experiment (Lisney *et al.*, 2011). In another study by Lisney et al. (2012), the CFF in two groups of different layers was assessed with electroretinograms (ERG) in several light intensities and compared with behaviourally assessed values. They found that ERG-derived values are higher, some individuals had ERG responses at 118-119 Hz. The chickens might not be able to consciously perceive flicker above 87 Hz, or else it should have shown in behavioural studies, but the ERG responses at flicker frequencies above 100 Hz means that poultry in artificial lighting might be able to resolve flicker even in fluorescent lighting (Lisney *et al.*, 2012).

Karlsson et al. (2009) had previously conducted studies where White Leghorns had poorer spatial learning capacity than its ancestor the RJF. They examined whether the Dominant white mutation in the PMEL17 gene, a mutation which gives the White leghorn its white feather plumage and which is known to cause visual impairment in other species, was the reason for this. But no differences were seen between chickens with or without the mutation in behavioural tests, where the chickens would discern a light stimulus from the background light, or from histological examinations of the eyes (Karlsson *et al.*, 2009).

2.2 Avian photoreceptors

The visual system of birds differ quite much from mammals but the width of the difference can be difficult to grasp. Birds have three types of photoreceptors, giving them a colour vision better than humans, and they can also perceive UV-light.

Birds possess one single type of rod in the retina and rods contain more of the pigment rhodopsin which absorbs more light than what cones do. Night active birds like owls therefore have more rods than cones to see better in dim light conditions, while day active birds have more cones for colour vision which consequently gives them less good night vision (Hart & Hunt, 2007).

There are four spectrally distinct classes of cones and one double cone in the retina among birds which gives them a tetrachromatic vision system that spans from 315 to 700 nm (Prescott & Wathes, 1999; Hart & Hunt, 2007). Connected to these cone cells are four colour pigments, compared to three of the human, which are responsible for colour vision during daylight. One of the colour pigments makes it possible for birds to perceive UV-light (Prescott *et al*., 2003; Hart & Hunt, 2007).

Birds also possess coloured oil droplets situated in the cone cells which filter incoming light that have shorter wavelength than the cones can perceive (Prescott *et al.*, 2003; Widowski, 2010). This is due to that all but one oil droplet contain carotenoid that absorbs short wavelengths of incoming light. Below the wave length of absorption, no light can be transmitted by the oil droplet. The peak sensitivity will shift upwards and make the birds' ability to discriminate hue more accurate (Prescott & Wathes, 1999). The oil droplet connected to the colour pigment which makes it possible for birds to perceive UV-light do not contain carotenoid and does not act as a filter for wave lengths in the UV-spectrum (Hart & Hunt, 2007).

2.2.1 The function of ultraviolet sensitive vision in birds

Day active birds have evolved to have two classes of colour vision concerning UV-light, violet sensitive (VS) and ultraviolet sensitive (UVS) (Ödeen & Håstad, 2013). The fact that the UVS visual pigment is present in many different bird orders that is not closely related suggests that there is some kind of advantage to have UVS colour vision (Hart & Hunt, 2007). In studies, it have been seen that inclusion of UV-light prevent feather pecking in turkeys and facilitate mating behaviour and mate choice in broiler breeder fowls as well as being important for the ability to detect rapid movements (Prescott *et al.*, 2003; Rubene *et al*., 2010). Birds with UVS colour vision can discriminate between more colours in their environment which can aid them in foraging and mate choice. When UVS birds have a feather plumage with colour patterns only discriminable in UV-light, they can be less detectable to VS predators but still stay visible for their conspecifics (Ödeen & Håstad, 2013). Vitamin D is produced by birds when they are exposed to UV-light outdoors, specifically UV-light in the range of 280-315 nm called UVB (ISO 2007). Vitamin D is important to strengthen the skeleton and to form egg shell, but no UVB-light can pass through window glass. Vitamin D is however added in the feed and it is unknown if there are other negative health concerns due to the lack of UV-light in poultry stables (Nilsson *et al.*, 2013).

There are a couple of known negative aspects of UV-light being transmitted to the retina. It can cause damage to the retina and UV-wavelengths are scattered by the ocular tissue which possibly makes the retinal image blurry. UV-wavelengths

that come from the atmosphere can therefore be a distraction to birds that must detect objects at long distances. Some birds, including chickens and layers, have the visual pigment that makes them VS which makes them less susceptible to the negative impacts of UV-light (Hart & Hunt, 2007). Conventional lighting in poultry production is normally not emitting any UV-wave lengths (Prescott *et al.,* 2003). In Sweden however, it is required to have UV-light included in the artificial lighting if it is necessary to keep out daylight due to animal welfare problems (SJVFS 2017:28).

2.2.2 The effect of light intensity and wave length on behaviour

Keeping poultry in low light intensity may deprive them of an important part of their sensory input since they are day active birds which see better in bright than dim light conditions (Manser, 1996; Hart & Hunt, 2007). A small study of ten layers showed that the motivation for eating was significantly higher in 200 lux compared to <1 lux (Prescott & Wathes, 2002), higher feeding motivation will also have a positive effect on production. However, it is considered likely that dim light intensities keep feather pecking at low levels. One cross-over experiment with 450 layers by Kjaer & Vestergaard (1999) compared keeping the layers in 3 lux or 30 lux with incandescent bulbs. The amount of gentle pecks was significantly higher in 3 lux and the amount of severe pecks was significantly higher in 30 lux at the first observation period at ten weeks of age. The mortality was significantly higher in the 30 lux group in the later period of the study, 16 to 46 weeks. Kjaer & Vestergaard theorized that the stereotypic, gentle feather pecking was developed to compensate for the sensory deprivation in the dim light condition. But they also stressed that feather pecking is a multi-factorial problem of which light intensity is only one of the factors.

Few studies of how chickens and layers react to different wave lengths have been conducted. One of the largest study was carried out by Huber-Eicher et al. (2013) with 600 layers in different colours from LED lamps. From 16 weeks of age, one group of the layers were brought up in red light (640 nm), the second group brought up in green light (520 nm) and the third group in white light. The layers in green LED showed more explorative behaviour by spending more time on foraging, pecking on conspecifics and pecking on objects compared to the layers in white and red light. Red light reduced aggressiveness compared white light (green light was intermediate), measured in distress calls and frequency of vigorous pecking. The layers in red light also showed a significantly better early laying performance of 70.6 % compared with 52.0 and 40.4 % in white and green light respectively. The laying performance were recorded by dividing the numbers of laid eggs during the last three days of the experiment with 75 (25 layers * 3 eggs) and multiplied by 100. So

red light could apart from reducing aggressiveness also be beneficial for accelerating sexual development in layers (Huber-Eicher *et al.*, 2013). Another preference test in different light colours was conducted by D'Eath & Stone (1999) in which they let eleven layers choose between eating in front of a familiar or an unfamiliar bird. The layers ate in front of a familiar bird most of the times in the white light and the least amount of times in the red light, the brightness of the light (77 or 5.5 lux) did not affect the result significantly. D'Eath and Stone (1999) hypothesised if the red comb and wattles were more difficult to distinguish for the layers in the red light, which are important visual cues to assess the social cues of unfamiliar birds. They also noted that their layers were raised in white light, if they had been raised in red light instead it could have been possible that they would learn how to recognize each other based on other visual cues instead.

The Swedish Board of Agriculture have in the legislation of animal welfare for layers (SJVFS 2017:28) stated that it is allowed to have other solutions than windows for incoming day light in stables for poultry. They further state that it is allowed due to animal welfare and health problems to have day-like artificial lighting instead of natural light as long as the spectral width is at least 300 nm and includes UV-light. The artificial light must also have a flicker fusion frequency of at least 120 Hz so it will not cause any distress for the poultry. One type of day-like artificial lighting that was tested in a new technique-trial for approval came from HATO®, carried out by Nilsson et al. (2013). Data of the production, growth rates, clinical health signs and behaviours connected to inappropriate lighting conditions were collected and analysed. The stables in the study was a single tier layer for young layers plus enriched cages for producing hens and an aviary system for producing hens. Most of the layers in enriched cages and aviary system had damages in the feather plumage but it could not be correlated specifically to the light conditions. It could also be seen that feather damages increased with the layers age which is consistent with how it usually is in the commercial production. No specific results from the clinical studies and behaviour tests showed any signs of impaired welfare due to the lighting conditions (Nilsson *et al.*, 2013).

2.3 Spatial resolution

Visual acuity is based on the smallest detail which can be detected in an object and also make up for a criteria level which can be used to assess the visual performance of an individual (National Research Council Committee on Vision, 1985). Acuity is determined by the density of photoreceptor cells and the size of the image projected onto the retina. Another term for acuity that can be used is spatial resolution (Prescott *et al.*, 2003).

Spatial resolution can be measured in an individual by determining at which thinness of gratings the test subject cannot discriminate a grated image from a uniform grey image. Gratings are based on three functions: spatial frequency, contrast and phase. Spatial frequency of the grating is the width of the black and white bands and it is measured as number of cycles (bars) per degree of visual angle (c/deg). A low spatial frequency has broad bands and a high spatial frequency consists of many thin black and white bands, see figure 1 for example.

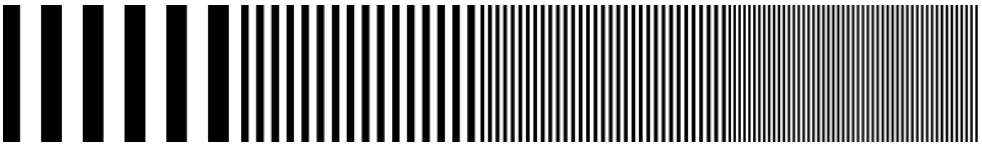

Figure 1. Demonstration of spatial frequencies. The gratings to the left represent a low spatial frequency while the gratings to the right show a high spatial frequency.

Figure 2 and equation 1 to 3 shows how to calculate the number of degrees per visual angle. To obtain the number of cycles per degree of visual angle, the number of cycles (black and white bars) (*x*) which can fit into an optional width of the stimuli is divided with the calculated degrees, see equation 4.

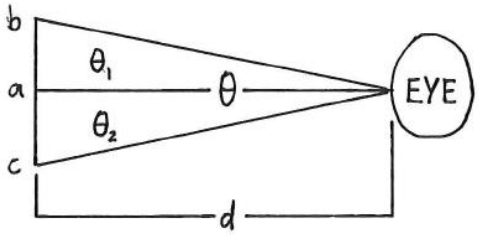

Figure 2. Visual angles for an object.

$$\tan\theta_1 = \frac{|ba|}{d} \quad (1.)$$

$$\theta_1 = \tan^{-1}\frac{|ba|}{d} \quad (2.)$$

$$\theta = 2 \times \tan^{-1}\frac{|ba|}{d} \quad (3.)$$

$$cdeg^{-1} = \frac{x}{\theta} \quad (4.)$$

Contrast is defined as the difference in luminance between an object and its background; for gratings it is the difference between the dark and light bars and varies

from 0 (no contrast) to 1 (full contrast). The phase of the gratings relates to how the black and white bands relate their position, e.g. vertical or horizontal, in relation to a certain point that is determined in advance, e.g. the lower left corner. A factor of the stimuli which affect the measured spatial resolution in the test subject is the illuminance of the grating (National Research Council Committee on Vision, 1985; Prescott *et al.*, 2003).

2.3.1 Spatial resolution threshold of chickens

Birds have a generally good spatial resolution due to their large eyes and consequently large retinal images (Diedrich & Schaeffel, 2009). Behavioural studies of chickens' spatial resolution have varying results and differ much in how they were executed.

Studies which have examined the spatial resolution threshold in chickens and layers have all had between five and eight subjects to base their results on. The chickens have ranged from being 12 h old as in Over & Moore (1981) to 12 months of age as in Jarvis et al. (2009). The housing of the chickens and light conditions prior to the testing have differed quite significantly. Gover et al. (2009) housed their layers outside with natural light after obtaining them at 16 weeks of age, up until then they had been reared under commercial conditions with unspecified light conditions. Jarvis et al. (2009) also kept their layers outside but they had complemented with artificial, fluorescent light to keep the luminance levels at 200 lux during the 16 h long light period, while Schmid and Wildsoet (1998) kept their chickens indoor with fluorescent light at 250 lux for a light/dark cycle of 12/12 h. Neither DeMello et al. (1992) or Over and Moore (1981) mentioned how they housed their chickens, but Over and Moore (1981) stated that their chickens were allowed normal visual stimulation throughout development without specifying it further.

The most common method to determine spatial resolution has been to use a behavioural response procedure. DeMello et al (1992), Gover et al. (2009) and Jarvis et al. (2009) trained their chickens to peck as a response when the stimuli was shown on computer screens or photographs while Over and Moore (1981) trained their chickens to jump to a grating which could support their weight while a homogenous field did not. Schmid and Wildsoet (1998) constrained their chickens, making them unable to move except from their heads, so they could follow the stimuli which were mounted on a rotatable arc at three different distances in front of the chickens. Their definition of the chickens being able to resolve the stimuli was that they followed the rotating stimuli with their eyes for at least 5 seconds of total 20 seconds of trial. Diedrich & Schaeffel (2009) differed from other studies with the same aim in that they determined spatial resolution *in vitro* by a microelectrode array. The chickens

in their study were decapitated and their eyes surgically removed. A part of the retina was cut out and placed on the array with the ganglion cells facing downwards against the electrodes. The stimuli were projected onto the retina through an artificial lens with a cathode ray tube monitor ("tjock-tv").

The most commonly used stimuli for measuring spatial resolution of chickens are gratings of varying spatial frequencies. Diedrich and Schaeffel (2009) on the other hand used a checker-board of varying sizes and grey values of the fields. The Michelson contrast used in the studies can be seen in table 1, it has varied between 0.78 (Schmid & Wildsoet, 1998) to 1 (DeMello *et al.*, 1992) or have not been specified. Diedrich and Schaeffel (2009) only said the contrast was kept as high as possible. The age and sex of the chickens used has also varied much which can also be seen in table 1. None of the studies have specified the spectral output of the lighting.

Table 1. *Details of the studies compiled in the literature review*

Study	Number of subjects and age	Michaelson contrast
Over & Moore (1981)	Not specified, 1-25 days	Not specified
DeMello et al. (1992)	Six females, age not specified	0.925-1
Schmidt & Wildsoet (1998)	Eight males, 2-8 days	0.78
Diedrich & Schaeffel (2009)	Five (retinas), 2-6 days	Close to 1 (not specified)
Gover et al. (2009)	Six females, 16 weeks	0.94
Jarvis et al. (2009)	Five females, 12 months	Not specified

The results of spatial resolution among chickens vary between the studies and have been illustrated in figure 3. In the results from the higher luminance levels, 1.79-57.35 cd/m^2, by Gover et al. (2009) the spatial resolution threshold of the chickens did not change much. The ability to maintain almost the same spatial resolution over a large range of luminance levels could according to Gover et al. (2009) have remained from the ancestor to the domestic chicken, the RJF, which roamed between different habitats of varying light intensities. Being able to remain the visual capacity when coming from one light intensity to another must have been an advantage in terms of surviving predators in the forests of Southeast Asia.

At the lowest luminance level, 0.06 cd/m^2, in the study by Gover et al. (2009) spatial resolution threshold was measured to 3.2 c/deg from only two birds that performed the task, the other showed roosting behaviour instead. A theory they had of why so few birds performed in dim light conditions could be that the control of rod function, i.e. the ability to absorb as much light as possible, is controlled by a strong diurnal force. The results obtained by Gover et al. (2009) were measured at daytime and this would then have the effect that the measured spatial resolution was not truly representing visual abilities under darker light periods.

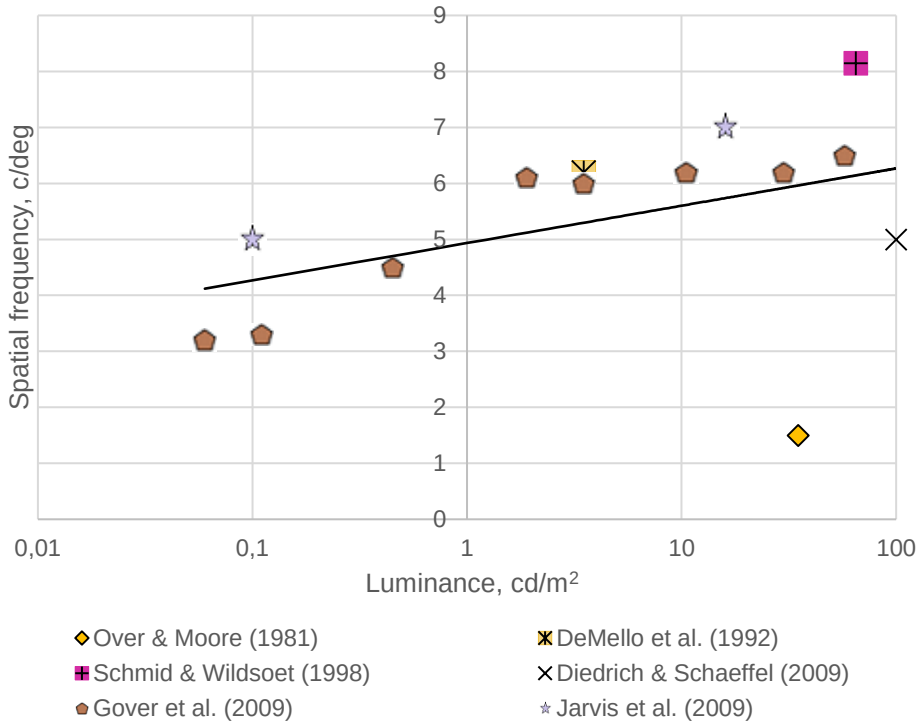

Figure 3. Spatial resolution threshold of layers as a function of luminance. Compilation of the result from earlier studies.

Because no one have studied how spatial resolution threshold is affected by light spectra or how the threshold differ between layers and its ancestor the RJF, the following trial was concerned with examining the effect of three light treatments on spatial resolution threshold among the two breeds using behavioural tests in a Skinnerbox.

3 Material and methods

3.1 Subjects and housing

The experiment took place at Lövsta research centre, Swedish University of Agricultural Sciences, Uppsala. A total of 192 chickens of the commercial layer breed Bovan Robust were brought to the facilities from a commercial hatchery (Swedfarm AB, Linköping) as day-old chicks. RJF eggs were brought to the research centre and approximately 30 of them hatched at the site in an incubator the same day as the layer chickens arrived. The RJF chickens came from a population which have been randomly bred for more than 12 generations at Linköping university (Zidar, 2017),. How the chickens looked like can be seen in figure 4. All chickens were housed indoors in pens with sawdust, heat lamp, water and commercial feed (Granngården) were available ad libitum throughout the whole study. As they got older, they also got access to perches and the tier floor. The layers were first divided into six groups of 30 chicks at arrival and then into twelve groups with sixteen chicks in each at four weeks of age. The 30 RJF chickens were first divided into two groups of fifteen chicks and later split into three groups of ten chicks.

Figure 4. The two breeds used in the study. A female RJF to the left, a male RJF in the middle and a layer of the breed Bovan Robust to the right.

Three types of experimental lighting were used in the experiment, one control light and two different experimental lights. All chickens were brought up in the control light for the five first weeks of age, thereafter the experimental lighting was installed. The experimental lighting was placed as centred as possible in the pens to gain as even light as possible. The experimental lighting was covered with two layers of white diffusion filters (Lee Filters, Andover) which lowered the light intensity with 20 % per layer of filter in order to get a similar illuminance of the home pens with control light. The mean illuminance in the pens were calculated to be between 5 and 7 lux when measured at floor level in all four corners and in the middle of the pen. The sides of the pens were covered with tarmac so the neighbouring pens lighting would not be visible. For the same reason, curtains of tarmac were hung up in the corridor so any light from other treatments would not be visible.

At the first days of age, the chickens had a 23 h dark and 1 h light period so they would learn to find food and water. The dark period was gradually extended with one hour each week so at six weeks of age and onward, the light period was 10 h and the dark period 14 h. The control light was gradually dimmed over 15 minutes at sunrise and sunset. The experimental lighting was turned off abruptly just before the control light started to decrease in light intensity, and turned on directly after the control light was fully lit.

3.2 Light treatments

Three light treatments were used in the study; control light, jungle light, and D65-light. The control light was LED light bulbs and the existing light at Lövsta research centre, see figure 5 for the graph for the light spectra of the lamp used in this study.

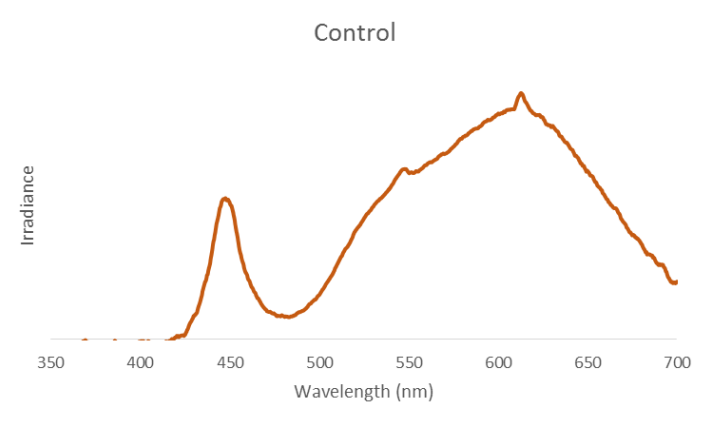

Figure 5. Light spectra of the control lamps.

The data for the jungle light spectra was collected in south-east India during September month in Tamil Nadu. The JAZ-S light spectrophotometer from Ocean Optics Inc. with OceanView software was used to measure the light spectra in the natural habitat of the RJF, natural and secondary growth dry deciduous forests, at sunrise, midday and sunset. The experimental lighting was then created by matching the amount of light received by each single cone type of the chicken between the natural measured light spectrum and a red-blue-green LED lamp, see figure 6 for the graph for the light spectra of the lamp used in this study.

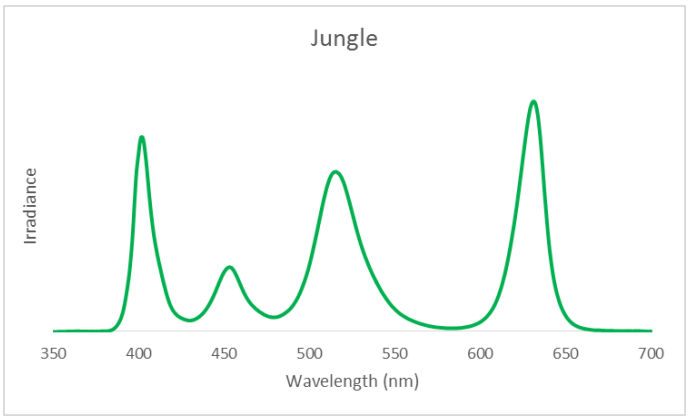

Figure 6. Light spectra of the jungle light lamps.

The D65-light is a standardized illuminant which is intended to represent average daylight according to International Commission on Illumination (ISO 11664-2:2007). In figure 7, the graph for the light spectra of the lamp used in this study is shown.

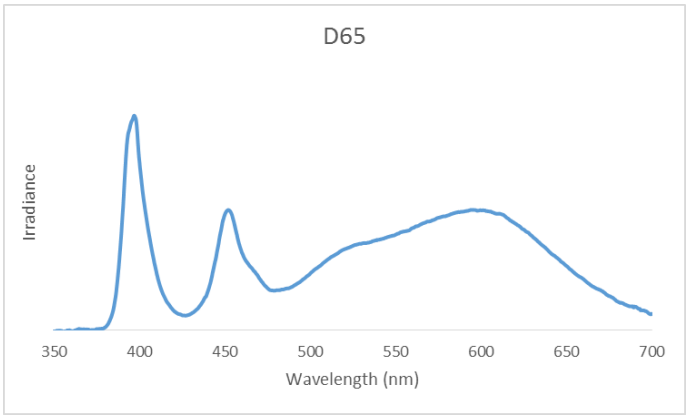

Figure 7. Light spectra of the D65-light lamps.

3.3 Experimental arena

The behavioural tests took part in a Skinnerbox which is shown in figure 8. It was constructed by walls of shuttering plywood with the measurements 110*65*59 cm and floor of medium-density fibreboard. At the shorter end of the box, two stimulus windows 8*8 cm were located, with a feed dispenser below each and a 30 cm dividing wall between them. A metal net covered the ceiling of the box and a flap door which could be lowered to hold the chickens at a distance of 60 cm was installed, attached to the ceiling. A hatch could be lifted or lowered to reveal or hide the stimuli and this was grey at start of training but was later covered with yellow plastic tape to have a better contrast for the chickens. Wood shavings on the floor were changed to a black plastic bag layer during training to make the environment less distracting for the chickens. The lamps were hanging in a wooden frame hung approximately 200 cm above the Skinnerbox.

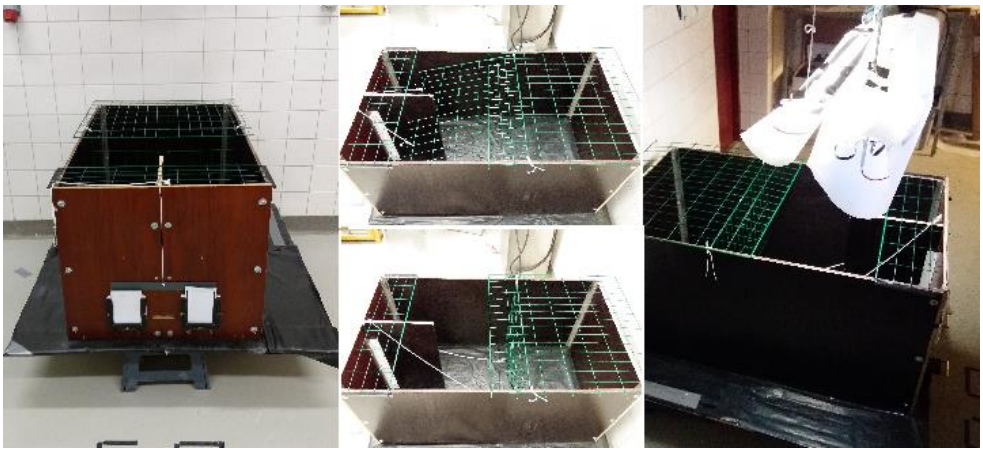

Figure 8. The Skinnerbox. To the left, viewpoint of the testarena. In the middle, the flap door is raised to allow the chickens to walk towards their assigned positive stimuli or lowered to keep the chickens at a distance of 60 cm. To the right, the experimental lighting is positioned above the Skinnerbox.

The stimuli were either a square-wave grating with vertical bars in the highest contrast setting possible or a grey, uniform stimuli of equal intensity calculated in GNU Image Manipulation Program (version 2.8.26) and printed on paper with a Canon MG7750 printer. The number of cycles of one stimuli picture was divided with the calculated degrees per visual angle, shown in equations 1 to 3, to obtain the number of cycles per degree (c/deg). The spatial frequency of a grated picture is depending on the distance of which the chicken chose to go toward a stimuli, i.e. if it was directly by the flap door or at the end of the dividing wall. If the chickens choose which side to go toward at the shorter distance, the spatial frequency was corrected for this. The correction resulted in approximately a halving of c/deg that the chickens could perceive, seen in table 2.

Table 2. *Spatial frequency of the gratings depending on cycle width and distance to the stimuli*

Cycle width (cm)	Spatial frequency (c/deg) at 60 cm distance	Spatial frequency (c/deg) at 30 cm distance
1,05	1,00	0,50
0,69	1,52	0,76
0,52	2,01	1,01
0,42	2,49	1,25
0,35	2,99	1,50
0,30	3,49	1,75
0,26	4,03	2,01
0,23	4,55	2,28
0,21	4,99	2,49
0,19	5,51	2,76
0,17	6,16	3,08
0,16	6,55	3,27
0,15	6,98	3,49
0,13	8,06	4,03
0,12	8,73	4,36

3.3.1 Experimental lighting

The light spectra of the LED jungle light was adjusted through the Wi-Fi controlled app Magic Home (version 1.3.3) by LED Controller. By connecting a smart phone to the Wi-Fi of the lamp it was possible to control the RGB-spectra of the jungle light through the app. The spectral output of the light treatments was measured with the same light spectrophotometer which was used in India, and the quantum catch for each cone type calculated and adjusted to match as closely as possible the jungle light and the D65 spectra.

The lamps with jungle light and D65-light were constructed by mounting LED- and UV-strips onto aluminium frames, see figure 9 for close up of construction. The amount of UV-light was additionally controlled indirectly by adjusting the light intensity and partly covering the UV-light strips with black electrical tape. White diffusion filters (Lee Filters, Andover) which could lower the light intensities by 20 and 40 % were used to obtain the different light intensities without lowering the settings too much so that the UV-light became too strong. The illuminance was also changed by lowering or elevating the lamp above the experimental arena. The illuminance was measured approximately 5 cm above the floor of the Skinnerbox, right below the lamp, before each training or testing session with the lux-meter Screen-Master from Hagner.

Figure 9. The construction of the experimental lights in the study, showing details of the LED-strips and the Wi-Fi-controller connected to the jungle light.

The birds were tested in four different illuminances (3.5, 10, 50 and 110 lux) in the Skinnerbox. These were measured with the lux-meter facing upwards in the centre of Skinnerbox and directly in front of the stimulus window with the lux-meter facing horizontally. To compare the results with previous studies, the luminance were also measured in front of the stimulus window with the lux-meter facing horizontally (cd/m^2).

3.4 Training procedure

The training of the chickens started at five weeks of age by habituation to the Skinnerbox. The training took part two to three times a week for each light treatment group. All groups were trained in the control light in approximately 10 lux for the first three weeks of training until the experimental lighting were available to use in the Skinnerbox. Groups of eight to ten chickens were put inside the Skinnerbox and were allowed to explore the Skinnerbox for 15 minutes for one to two days. When the chickens seemed to be more relaxed when exploring the Skinnerbox, the feed reward (live meal worms) was put on top of the feed dispenser situated in the front end, facing the operator, of the Skinnerbox to draw the attention of the chickens toward the feed dispenser. Once the chickens knew where to find the reward, the hatch of the feed dispenser was opened to reveal more food inside. By opening the hatch each time a chicken pecked on the feed dispenser to eat a meal worm, they learned how to retrieve food when it was hidden inside the feed dispenser. Feed was hidden inside the feed dispensers of both sides of the dividing wall.

Once the chickens had learnt to go toward and peck on a feed dispenser in order to retrieve a reward, the stimuli was introduced (se figure 10 for example of how the stimuli presentation would look like from the chickens' perspective). Three groups with four layers in each group, one from each light treatment, were randomly assigned to test positively on one of the stimuli. The three RJF groups of four chickens were divided within each group into two pairs, each of the pairs were randomly assigned to test positively to one of the stimulus. To avoid any positional preferences

of the chickens, the stimuli swapped positions in the front end of the Skinnerbox randomly so that they were presented an equal number of times to the right or left side of the dividing wall, but no more than two times consecutively on each side. When the stimuli were introduced, the chicken would only receive feed when it pecked on the feed dispenser connected with the assigned positive stimuli. When the chickens went to the wrong stimuli, the flap door was slowly lowered, forcing them to return to starting position at the end of the dividing wall, from where they could go to the other feed dispenser. After doing so for some training sessions (the number of sessions varied due to the different levels of motivation of the groups but were approximately between two to ten sessions) they learned that feed could only be retrieved in the feed dispenser situated below their assigned stimuli.

Once they had learnt to retrieve food below their assigned stimuli, a protocol of the result were filled in for each chicken. When the chickens scored 75 % correct choices out of twelve trials, the tests started.

Figure 10. Stimuli presentation in the Skinnerbox. The left picture shows the stimulus windows when the hatch covered the stimulus. The right picture shows an example of a stimuli of 1 c/deg compared to the uniform grey picture.

3.5 Testing procedure

Each test started with a chicken placed in the far end of the Skinnerbox and the flap door lowered. The stimuli were presented randomly on each side of the dividing wall but not more than two times on the same side in a row, as during the training period. The stimuli were shown to the chicken for approximately five seconds before the flap door was raised in order to let the chicken have time to see the stimuli on both sides of the dividing wall. The flap door was raised, the chicken could approach their chosen stimuli and receive a food reward if the choice was correct. It was noted if the chicken walked directly towards the stimuli window or if the paused

or switched the direction at the dividing wall then the stimuli windows were shut, the flap door lowered again and the chicken returned to the starting position. The test were started at 2, 3 or 4 c/deg depending on the light intensity tested (3.5, 10, 50 or 110 lux). The stimulus were presented with increasing spatial frequency. When the chicken could not perceive a higher frequency of a stimuli, the test was finished and the spatial frequency of which the chicken obtained at least 70 % correct choices out of ten trials was considered as the highest frequency that the chicken could perceive. The test procedure is illustrated in figure 11 and this was performed for all chickens in all four light intensities.

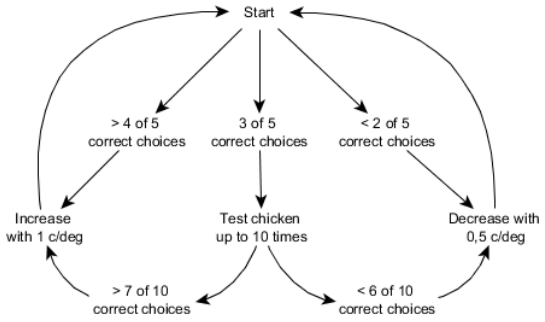

Figure 91. Testing procedure.

3.6 Statistical analyses

The results were analysed with One way ANOVA tests in MS Excel with the additional Analysis Toolpack to test for differences in spatial resolution threshold between breeds, between light treatments and between light intensities. Separate analyses was conducted for comparisons between layers and RJF within each light treatment as well as comparisons between light treatments and within each breed. A Least Significant Difference (LSD) test was conducted when results from the light treatment comparison within breeds were significant. The groups of RJF which had both males and females were not treated differently in the statistical analysis since the number of chickens was relatively small. To make the statistical analysis easier to conduct and to get a better overview, the chickens result in the four different light intensities were combined into one set of data per breed in each table. All tests had α-level of 0.05.

4 Result

The results from all 24 chickens included in the study, in all light intensities, are compiled in table 3. A number in the table represents the spatial resolution threshold of one chicken in a specific light intensity and light treatment. All chickens were able to show their individual spatial resolution threshold at each light intensity. One layer from the control light group and one from the jungle light group, the ones with the lowest scores, was excluded from the result in order to get balanced groups for the statistical analysis.

Table 3. *Spatial resolution threshold (c/deg) for all chickens in the study*

Light intensity	Control light		Jungle light		D65 light	
	Layer	RJF	Layer	RJF	Layer	RJF
3,5 lux	3,50	2,63	5,69	2,00	0,88	5,57
	3,50	3,00	3,38	2,36	2,19	3,00
	3,75	3,00	3,50	2,63	3,00	6,30
	5,63	2,50	3,50	2,50	5,18	4,22
10 lux	3,71	5,25	6,04	4,50	1,88	4,95
	3,76	7,00	2,63	2,63	5,40	2,50
	3,00	2,50	5,00	3,75	3,50	8,44
	5,69	1,25	4,50	4,64	2,40	4,50
50 lux	3,94	3,00	2,25	5,00	3,06	9,00
	3,75	7,43	4,69	4,38	5,50	4,00
	4,81	3,86	5,50	3,38	3,00	6,75
	4,47	2,19	3,75	3,43	4,50	3,00
110 lux	3,44	3,06	4,81	4,50	4,00	6,13
	4,64	4,00	3,38	7,00	6,00	3,00
	3,75	3,75	4,88	4,50	3,50	6,00
	4,13	2,63	6,25	3,50	6,00	3,00

4.1 Comparisons between the breeds in different light treatments

Comparisons between layers and RJF and the One-way ANOVA analyses of them in each light treatment group are described below. In the analysis, the spatial resolution thresholds of all chickens in all light intensities were added together, while the mean spatial resolution threshold of each light intensity is shown in the figure to illustrate how the threshold increase with illuminance. The detailed ANOVA analyses can be seen in appendix 1.

The mean spatial resolution threshold for layers and RJF across all light intensities in the control light treatment (figure 12) were 4.092 vs 3.566 c/deg respectively, a non-significant difference (p-value > 0.05).

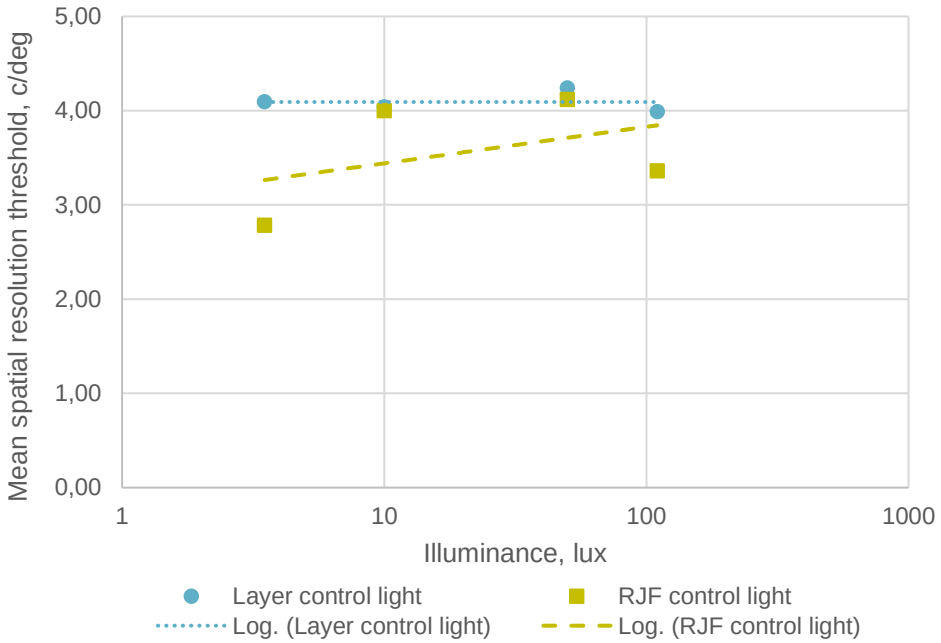

Figure 10. Comparison between breeds in the control light treatment as a function of illuminance.

The mean spatial resolution threshold for layers and RJF across all light intensities in the jungle light treatment (figure 13) were 4.359 vs 3.794 c/deg respectively, a non-significant difference (p-value > 0.05).

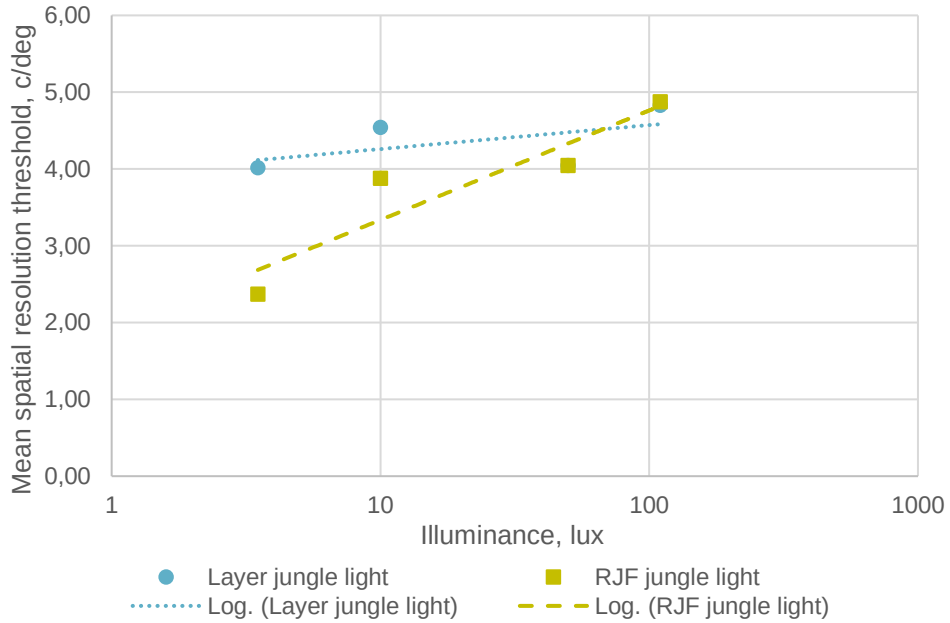

Figure 11. Comparison between breeds in the jungle light treatment as a function of illuminance.

A near significant result was obtained in the comparison between layers and RJF in D65-light (p-value 0.053), the mean spatial resolution threshold for layers and RJF across all light intensities were 3.749 vs 5.023 c/deg respectively (figure 14).

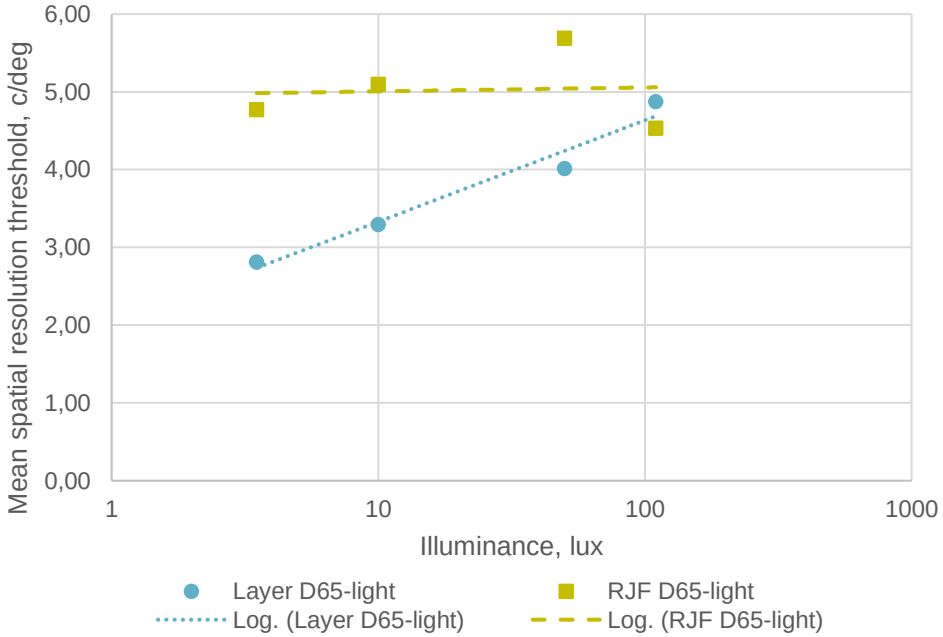

Figure 12. Comparison between breeds in D65-light treatment as a function of illuminance.

4.2 Comparison between light treatments for layers and RJF

Comparisons between light treatments and within breeds are described below. In the analysis, the spatial resolution thresholds from all chickens in all light intensities were added together, while the mean spatial resolution threshold of each light intensity is shown in the figure to illustrate how the threshold increase with light intensity. The detailed ANOVA analyses can be found in appendix 1.

The mean spatial resolution threshold across all light intensities were similar in the comparison of layers (figure 15). The jungle light group had a slightly higher but non-significant mean (p-value > 0.05) at 4.359 c/deg compared with the D65-group who had the lowest mean with 3.749 c/deg while the control light group were intermediate at 4.092 c/deg.

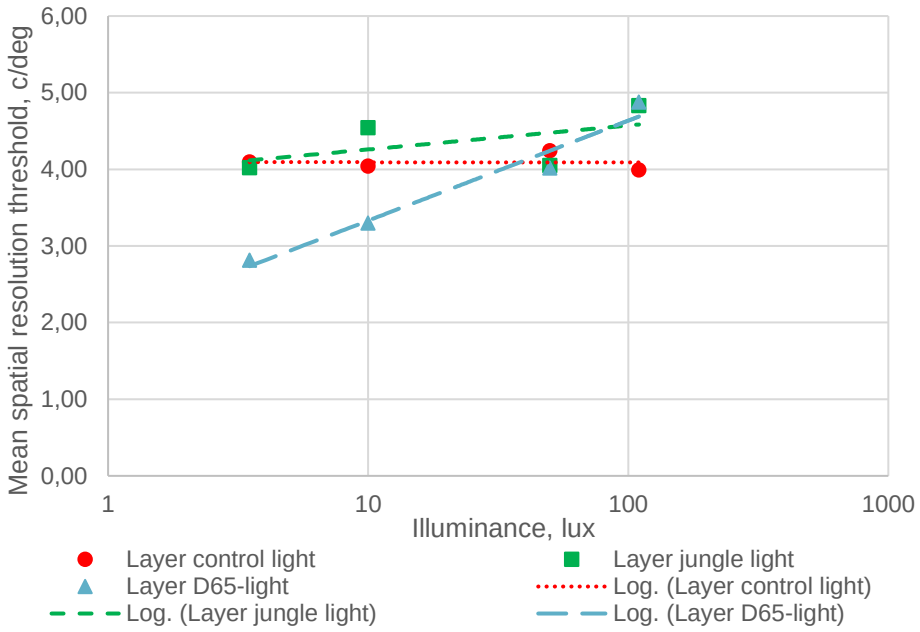

Figure 13. Comparison of spatial resolution thresholds between the light treatments and layers as a function of illuminance.

A significant result was found for RJF (p-value < 0.05), the D65-group had a mean spatial resolution threshold of 5.023 c/deg across all light intensities compared with the lowest threshold of 3.566 c/deg in the control group and the intermediate jungle light group 3.794 c/deg (figure 16).

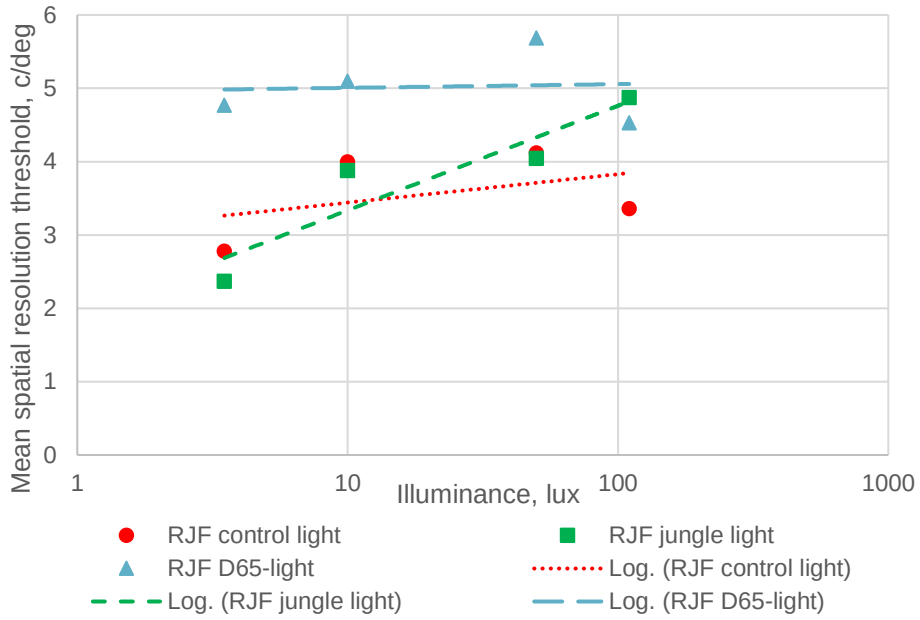

Figure 14. Comparison of spatial resolution thresholds between the light treatments and RJF as a function of illuminance.

4.2.1 LSD test of light treatment comparison within RJFs

To see how the RJF groups differed, a LSD test was conducted. The test showed that the group of RJF in the D65-light treatment had a significantly higher spatial resolution threshold from the other groups.

$$\text{LSD} = t_{0.95,,N-a}\sqrt{MS_e\left(\frac{1}{n}+\frac{1}{n}\right)} = 1{,}684\sqrt{2{,}8\left(\frac{1}{16}+\frac{1}{16}\right)} = 0{,}996$$

A1: control light
A2: D65-light
A3: jungle light

$$\text{A1} - \text{A2} = 3{,}566 - 5{,}023 = |-1{,}457| > 0{,}966$$

$$\text{A1} - \text{A3} = 3{,}566 - 3{,}794 = |-0{,}228| < 0{,}966$$

$$\text{A2} - \text{A3} = 5{,}023 - 3{,}794 = 1{,}229 > 0{,}966$$

4.3 Comparisons between breeds in low light intensity

No significant result was found between breeds when all results of spatial resolution thresholds per breed were analysed in the same One-way ANOVA test. But when

the analyses of the results in the lowest illuminance were separated by light treatment in figure 17, significant results was found in the control light (p-value < 0.05) and jungle light (p-value < 0.05) groups. In both those groups, the layers had a higher mean spatial resolution threshold. The control light layers had a spatial resolution threshold 4.095 c/deg and the jungle light layers 4.017 c/deg compared with 2.783 and 2.373 c/deg among the RJF in respective light treatment group. The comparison between the breeds in D65-light were non-significant (p-value > 0.05), the mean spatial resolution threshold of the layers were 2.813 c/deg and 4.773 c/deg among the RJF. The detailed ANOVA analyses can be found in appendix 1.

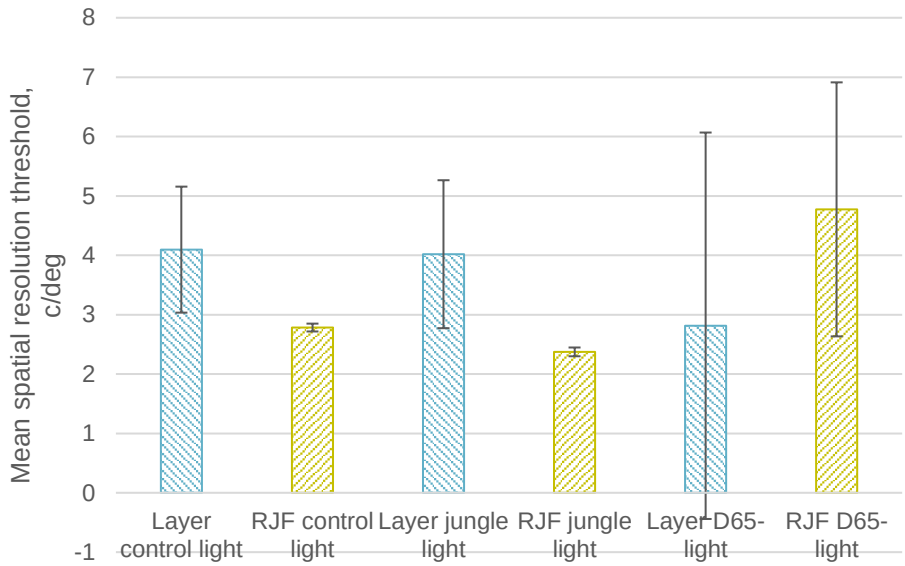

Figure 15. Comparison of spatial resolution thresholds between the light treatments and the breeds in the lowest illuminance of 3.5 lux.

5 Discussion

As mentioned earlier, the lighting regimes effect on chicken behaviour and production have been well researched but the effect of the spectral composition is not as much explored. This was the specific aim of this thesis, to see how visual abilities of chickens are affected by different light spectra. In the next paragraphs, I will try to answer the specialized questions from the introduction.

Will the spatial resolution threshold differ between the RJF and the white layers in different light intensities since the layers have been bred and selected for egg production in stables with low light intensities for decades?

No significant differences were found between the two chicken breeds when the spatial resolution thresholds in all four illuminances for one breed were added together in the ANOVA analyses and compared with the results of the other breed in the same light treatment. The only near significant result was found in the comparison between the breeds in D65-light (p-value 0.053) in which the RJF were a little better. Since the question further wondered if there are any differences between the breeds since layers have been kept and bred in low illuminance for a long time, one separate set of analyses was made with only the results from 3.5 lux. In those analyses, the layers had higher spatial resolution threshold in the control light (4.095 vs 2.783 c/deg, p-value < 0.05) and jungle light (4.017 vs 2.373 c/deg, p-value < 0.05) respectively. Commercial layers have been reared and kept in stables with low illuminance for many decades to, among several reasons, keep stress symptoms on a low level (Manser, 1996). The ancestors of the modern layer, the RJF, lives in a habitat in which the light varies each day and has seasonal changes. When it becomes dark the RJF rests in the trees (Collias & Collias, 1967) and have not been forced to search for food or interact with other birds during dim light conditions like layers have. If the layers have adapted their visual capacity to see better in low illuminance, without any specific breeding strategy from the breeding companies, it could be an answer to why there was significant differences in the 3.5 lux comparisons between breeds. The significant result may be seen as an evidence for layers

being able to see better in dim light conditions which is occurring in commercial layer facilities. It would be interesting to see if a study with a larger dataset would get the same result. Another reason explaining these results could be that the RJF were more stressed in the test situation than the layers and not performing their best.

Will the spatial resolution threshold change for the chickens if UV-light spectrum is included in the light sources?
Adding UV-light in the light treatments (D65 and jungle light) do not seem to have resulted in any different spatial resolution threshold between the treatments. The only significant result from a light treatment with UV-light was from the comparison between the groups of RJF, where the D65-group had the highest spatial resolution threshold of 5.023 c/deg (p-value < 0.05) compared to the control light (3.566 c/deg) and the jungle light (3.794 c/deg). However, D65 and jungle light contained similar amount of UV light, so this difference is unlikely because of more UV in the D65 treatment. D65 contained more blue light than control and jungle light and this type of light dominates in open habitats under blue sky (Endler, 1993), e.g., forest edges and clearings where jungle fowl forage. So it is possible that higher spatial resolution is an advantage when searching for food under these light conditions.

Will the light spectrum that simulates the light conditions found in the lower vegetation in Southeast Asia affect spatial resolution threshold of the chickens?
The jungle light seem to have not been different from the other light treatments in any of the analyses but the comparison between breeds at 3.5 lux. The layers had a mean spatial resolution threshold of 4.017 compared to 2.373 c/deg of the RJF (p- value < 0.05) in the lowest illuminance. The jungle light represents the light environment in the forest interior which is a roosting environment for the jungle fowl during night, with lower light levels than the foraging habitat. It could have been expected that the spatial resolution would be higher at low light intensities in these condition to help jungle fowl navigate the forest environment during dawn and dusk.

5.1 Comparison to previous studies

It is difficult to compare the result from this thesis with previous research on the same topic since the methods and subjects differ so much. This is the first study of spatial resolution threshold that I am aware of that has light spectra as an aspect to take into consideration and which compare commercial layers with its ancestor the RJF in several light intensities and in light intensities as low as in this study. Other

studies have had between five and eight chickens to test, we had a total of 24 chickens.

One thing which is possible to compare with other studies is the spatial resolution as a function of luminance. All other studies in the field have measured light intensity as luminance in cd/m^2 while this study measured illuminance in lux. For this purpose the incoming light to the stimuli was also measured as luminance in cd/m^2.

This is the first study to measure spatial resolution threshold in luminance as low as this. The results in this study will hopefully contribute with new knowledge and understanding for how spatial resolution threshold varies over light levels.

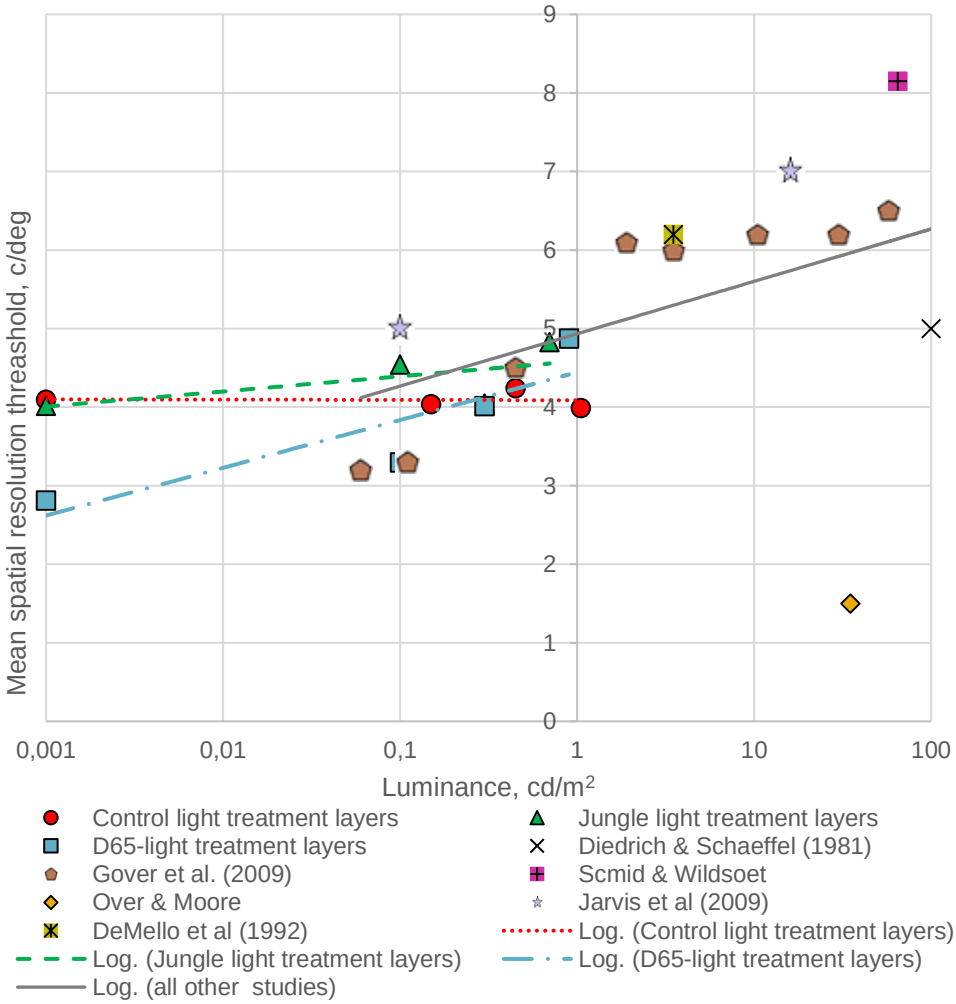

Figure 16. The results from this study compared with previous studies as a function of luminance.

As stated previously, none of the previous studies of spatial resolution threshold among chickens have specified the spectral output of their type of light other than

mentioning that fluorescent light was used (Schmid & Wildsoet, 1998). As seen in figure 18, the trend line of the D65 light treatment layers is almost perfectly aligned with the trend line which is fitted to all results from previous studies. Meanwhile, the trend lines fitted to the results of the control and jungle light treatment layers shows higher values of spatial resolution threshold in the lower light intensities. Gover *et al*. (2009) discussed whether there could be any visual impairment in their study due to the spectral characteristics of the light they used (fluorescent lighting). When comparing the trend lines for the different light treatments in figure 17, it may seem that there is such an impairment in the other studies result. The spectral output of the jungle and control light in this study seems to result in better visual abilities in the lowest light intensities.

5.2 Discussion about the used methods

The fairly low number of tested chickens is probably the reason of why most of the result is non-significant and with high variance. The original plan to test eight chickens per breed and light treatment showed to be undoable in the time given, therefore the number of chickens was halved. It seems unlikely that the spatial resolution threshold could be correlated to the trainability of the chickens. Because of that, the chickens which seems most easy to train, i.e. showed the lowest levels of stress, were chosen to continue the training. Due to the fairly low number of chickens, the results are not possible to generalize to a bigger context. But compared to the other studies conducted in the area, with five to eight test chickens in total, the number of test chickens in this study is high.

All chickens were brought up in the control light for the first five weeks of age before the experimental lighting was installed. Over and Moore (1980) and Schmid and Wildsoet (1998) reported that they saw a peak in spatial detail sensitivity in a very young age in their chickens (2-8 days). If the visual system of chickens could be affected by the spectral output in such an early age, it may be that rearing the test chickens in the control light have influenced the results.

Only one lamp was used for testing the jungle and D65-light groups, so to test the chickens in the different light intensities the lights had to be calibrated before each session. To save time and make the risk for calibrating the light wrong, separate lamps for each light intensity had been safer but this was not possible due to budget. An alternative would have been to test the chickens in fewer light intensities, which could have given more time to train and test more chickens.

Gover *et al.* (2009) had a 30 minute long adaptation period for their birds prior to testing and they said that the dim light conditions probably induced drowsiness

in the birds resulting in low motivation for participation in the lowest light intensities. The chickens in this study were kept in a transportation box with air holes between catching and testing and could be in there for more than 30 minutes some days. Despite that, the motivation in my chickens was quite high, resulting in having spatial resolution thresholds for all chickens in this study. An important part in this probably has to do with the live mealworms being the feed reward in comparison with boiled maggots that were used by Gover *et al*. (2009).

It was not possible to differentiate males from females while the RJF chickens were small during the beginning of habituation and training. When the final decision about which chickens to continue training with came, the sexes were more different from each other. I choose the chickens which seemed to have the most talent or understanding of the task and I tried to have two each of females and males to even out eventual differences between the sexes. To rule out any kind of sex difference it would have been better to only use female jungle fowls to compare with the female layers.

The routine of catching, training and testing could be the same every day in almost all aspects which made the chickens feeling secure during handling. If some part of the procedure differed from one time to another, the RJF got more distressed over it. The RJF is known to be more fearful than domesticated breeds of chickens (Agnvall *et al.*, 2012) so this difference between the breeds were no surprise. Some examples of changed routines regarding the Skinnerbox could be if I got up to bring more meal worms to the feed dispensers or needed a new protocol paper for a chicken. The testing procedure were conducted in a separate room but the personnel doing the daily care routines in the stable could sometimes make loud noises, which could be heard and disturb the chickens. It is difficult to take care of all the other chickens without making disturbing noises, so if it would have been possible to train and test chickens during hours when personnel were not there it could have minimized the number of cases with stressed chickens.

The chickens had ad lib feeding in their home pens and there is a risk that the free access to feed have affected the results. If the chickens had just eaten before the testing procedure, they would maybe not have been motivated to work which would have resulted in a seemingly low spatial resolution threshold. White Leghorn layers have shown to be less motivated to access food (Lindqvist & Jensen, 2009) so it is plausible that this could be the same for the layers in this study. Another concern that arose during testing were if the chickens would choose the wrong on purpose to reach a feed dispenser with more mealworms in it. Since the chicken got easily interrupted, especially the RJF, I did not pause every testing session to fill up the feed dispensers. If one feed dispenser were emptier than the other, resulting in less sound from the worms inside, the chicken would have a reason to be more motivated to choose wrong side. In the end of the study, I had a smaller box of mealworms

right next to me so I could fill up the feed dispensers with less disturbance to the chicken inside the Skinnerbox.

Lisney *et al.* compared CFF obtained with ERG (2012) and behavioural tests (2011) and found out that ERG derived values were higher, i.e. the chicken retina were able to detect flicker at higher frequencies than measured before. A lack of motivation for the task is difficult to differentiate from the chickens actually having a low spatial resolution threshold. Measuring a physiological feature with behavioural observations may have this as a risk.

5.3 Differences between the breeds and sexes

No significant statistical difference was found in the comparisons between breeds. A difference in behaviour between them was however noted. The layers were less frightened in human presence throughout the whole training and testing procedure than the RJF, in consistence with other behavioural studies about the RJF (Andersson *et al.*, 2001; Lindqvist & Jensen, 2009; Agnvall *et al.*, 2012). The RJF were more easily interrupted by unforeseen happenings while they were in the Skinnerbox and could less often continue the training and testing after such events compared to the layers. A reason why the D65-group of RJF had better results than the other light treatment groups, could be that they had less interruptions during training and testing. The RJF seemed to have more difficult to understand that thinner stripes of the stimuli (higher spatial frequencies) were the same stimuli that they should test positive on. The stimulus were presented with increasing frequency and if I skipped to many spatial frequencies of the stimuli during testing (for example, going directly to 6 c/deg from 2 c/deg instead of taking 3, 4 and 5 c/deg first), they did not get good results. So I generally needed take it more stepwise with the RJF, but the risk was that they got tired of the longer testing procedure and showed a lower spatial resolution threshold because of that instead. Studies about spatial learning differences between layers and RJF have found signs of the opposite, that layers have lower learning capacity (Karlsson *et al.*, 2009; Lindqvist & Jensen, 2009). The tests in this study could contain more physical contact and presence of humans, making the RJF more stressed and less susceptible to training.

A small difference between sexes among RJF could also be noted. The male jungle fowls was a bit more difficult to catch since they had a more explosive and protective temperament. They were on the other hand a bit faster to learn during training than the females. But individual differences were seen in both sexes, some females were more difficult to handle or easier to train than the males.

The layers came from the commercial hatchery Swehatch in Linköping. The breeding of commercial layer hens is very intensive and is mainly focusing on high

egg production. The RJF chickens came from a population held at Linköpings University and is in a breeding program in which they are bred randomly. This have resulted in a larger variation in the phenotype of the RJF compared to the layers, which could suggest that the layers also might have a more similar visual capacity. This could possibly mean there is a larger variation in spatial resolution thresholds among RJF chickens. The parents and grandparents of the RJF in this study have not always been kept in the same type of light environment which would be found in their natural habitat and may therefore have had the possibility to adapt to artificial lighting. This group of RFJ should nevertheless be much more genetically similar to the wild RJF and therefore have a more similar visual capacity to them than to the layers.

5.4 Implications to the commercial poultry production

The breeding of layer hen hybrids is performed by a few international companies which supplies the Swedish stocking agents with the parent animals of layers. The parent animals are crossbred with each other, resulting in the producing animals which in turn are transported to the egg producer at 15 weeks of age (Svenska ägg, 2017). The elite animals bred abroad could have other legislations of spectral output (or none at all). So if visual abilities are linked to any selected traits, it might therefore have been affected by artificial selection. If they have adapted to a certain spectral output which is commonly used in that country, it would certainly affect the production animals here in Sweden and the layers in this study. There were some statistically significant results between the layers and RJF found in this study, and it have been previously shown that there are visual differences between commercial layers, game breeds and RJF (Lisney *et al.*, 2011; Roth & Lind, 2013).

Using LED as a light source seems promising when reading Nilsson et al. (2013), who found no incidence of impaired (nor improved) welfare in the stables which had LED. LED is an energy-saving, low maintenance, long life light source with high reliability and it is possible to adjust wavelength (Huber-Eicher *et al.*, 2013). The possibility to adjust wavelength in LED should be interesting for the layer business since it seems possible that the behaviour of layers can be directed, to be more explorative, to reduce aggressive behaviour and also to increase laying performance (D'Eath & Stone, 1999; Huber-Eicher *et al.*, 2013).

If these results were possible to generalize to a larger scale, it would have seemed like the spectral output in this study's light treatment does not have a statistical significant effect for the layer visual abilities in general. The spatial resolution threshold is almost the same for the layers with the control and jungle light treatment. But when looking at the mean spatial resolution threshold at the lowest light intensity,

there is a statistically significant higher mean in the jungle light treatment. The trend lines plotted to the mean thresholds shown in figure 17 clearly shows there is a difference in the lowest light intensities.

5.5 Future directions

To deepen the understanding of how the spatial resolution threshold is affected by different light spectra, visual tests should be performed in one colour at a time. But since it takes so long time to train chickens how to perform the testing procedure in a Skinnerbox, it may be more effective to do behavioural studies measuring feather pecking incidence, foraging behaviour, egg production etcetera. When a layer hen is producing and feeling well, it should be unlikely that her visual capacity is reduced due to the light conditions.

It would be interesting to see a larger behavioural test with feather pecking incidence and cannibalism in the jungle light spectra due to the higher spatial resolution threshold in low light intensity in that light treatment. Then it would be possible to see if feather pecking and cannibalism is inhibited by low spatial visual capacity caused by low light intensity or if it is correlated to something else.

6 Conclusion

The spatial resolution threshold among the layers and RJF were different depending on which light spectra they were housed in and tested with. There was no difference between the breeds, apart from the results in the lowest illuminance in which the layers in the jungle and control light treatments had higher spatial resolution threshold. The thresholds were also higher when compared to results in previous studies with the same topic. These results implicates that the spatial resolution threshold of layers in low light intensities can be improved with the spectral output of the jungle light used in this study.

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Appendix 1

Comparison between the breeds in different light treatments

Control light comparison between breeds

Groups	Count	Sum	Mean	Variance		
Layer	16	65,47	4,092	0,589		
Red jungle fowl	16	57,05	3,566	2,824		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	2,216	1	2,216	1,298	0,264	4,171
Within groups	51,19	30	1,706			
Total	53,41	31				

Jungle light comparison between breeds

Groups	Count	Sum	Mean	Variance		
Layer	16	69,75	4,359	1,446		
Red jungle fowl	16	60,70	3,794	1,623		
ANOVA						
Sources	SS	df	MS	F	p-value	F-crit
Between groups	2,559	1	2,559	1,668	0,206	4,171
Within groups	46,03	30	1,534			
Total	48,59	31				

D65-light comparison between breeds

Groups	Count	Sum	Mean	Variance		
Layer	16	59,99	3,749	2,410		
Red jungle fowl	16	80,36	5,023	3,953		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	12,97	1	12,97	4,070	0,053	4,171
Within groups	95,59	30	3,186			
Total	108,6	31				

Comparison between the light treatments for layers and red jungle fowls

Layer comparison between light treatments

Groups	Count	Sum	Mean	Variance		
Control light	16	65,47	4,092	0,589		
D65-light	16	59,99	3,749	2,420		
Jungle light	16	69,75	4,359	1,446		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	2,992	2	1,496	1,007	0,373	3,204
Within groups	66,81	45	1,485			
Total	69,81	47				

Red jungle fowl comparison between light treatments

Groups	Count	Sum	Mean	Variance		
Control light	16	57,05	3,566	2,824		
D65-light	16	80,36	5,023	3,953		
Jungle light	16	60,70	3,794	1,623		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	19,65	2	9,825	3,509	0,029	3,204
Within groups	126,0	45	2,800			
Total	145,6	47				

Comparison between breeds in low light intensity

Low light intensity control light comparison between breeds at 3.5 lux

Groups	Count	Sum	Mean	Variance		
Layer	4	16,38	4,095	1,061		
Red jungle fowl	4	11,13	2,783	0,066		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	3,445	1	3,445	6,114	0,048	5,987
Within groups	3,381	6	0,563			
Total	6,826	7				

Low light intensity jungle light comparison between breeds at 3.5 lux

Groups	Count	Sum	Mean	Variance		
Layer	4	16,07	4,017	1,246		
Red jungle fowl	4	9,490	2,373	0,074		
ANOVA						
Source	SS	df	MS	F	p-value	F-crit
Between groups	5,412	1	5,412	8,199	0,029	5,987
Within groups	3,961	6	0,660			
Total	9,373	7				

Low light intensity D65-light comparison between breeds at 3.5 lux

Groups	Count	Sum	Mean	Variance		
Layer	4	11,25	2,813	3,254		
Red jungle fowl	4	19,09	4,773	2,139		
ANOVA						
Source	SS	Df	MS	F	p-value	F-crit
Between groups	7,683	1	7,683	2,849	0,142	5,987
Within groups	16,18	6	2,696			
Total	23,86	7				