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Light environments for dairy cows

Impact of light intensity, spectrum and uniformity

Sofia Lindkvist

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Sofia Lindkvist
Faculty of Veterinary Medicine and Animal Science
Department of Nutrition and Management
Uppsala

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Cover: Cow walking through the obstacle course in darkness, captured with a thermal imaging camera (photo: K. Ståhlberg)

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Swedish University of Agricultural Sciences, Department of Animal Nutrition and Management, Uppsala, Sweden

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Light environments for dairy cows. Impact of light intensity, spectrum and uniformity

Abstract

Light can be used as a management tool to increase milk yield in dairy cows and improve the working conditions for barn staff. It is known that a long day photoperiod, with 16 hours of light and 8 hours of darkness, can increase milk yield in an ongoing lactation. Modern LED lighting can be designed to emit specific wavelengths, opening up possibilities for discussing the most favorable type of light for dairy cows. This thesis investigated the role of light environment and the impact of light intensity, spectral composition and uniformity on dairy cows. In initial studies, a light lab with a controlled light environment and no external light was used. The response to red, blue, and white light of increasing intensity on pupil size was evaluated in five pregnant non-lactating cows. Red light did not constrict the pupil but the other light colors did, indicating that direct stimulation of ipRGCs may be required for a pupillary response to steady background light. A five-week study on 40 pregnant and lactating cows involving 16 hours of blue, red or white light in daytime and 8 hours of dim, white light at night did not show effects of light color during daytime on milk production. Plasma melatonin concentration was higher in dim night light than in daylight for all light treatments. To examine cow movements in light of different intensity, spectrum and uniformity, 12 pregnant, non-lactating cows were tested in an obstacle course in the light lab. A dark environment did not limit the cows' ability to walk through the obstacle course, but they reduced walking speed when subjected to non-uniform, low-intensity red light, indicating the importance of avoiding non-uniform light in dairy barns. Quantification of light environments on four Swedish dairy farms, using a range of measuring methods, showed that the light environment differed between farms, but that light of low intensity and uniformity was commonly used. Light environment is important for dairy cows, as it can affect their physiology and behavior. The light environment can be more objectively described using multiple measuring methods.

Keywords: Milk production, night light, dim light, arena test, walking behavior, pupillary response, melatonin, IGF-1, obstacle course

Ljusmiljöer för mjölkkor. Inverkan av ljusintensitet, spektrum och uniformitet

Sammanfattning

Belysning kan användas som ett verktyg för att öka mjölkavkastningen hos mjölkkor och förbättra arbetsförhållandena för ladugårdspersonalen. Ökningen i mjölkmängd är väl beskriven i studier där den naturliga dagen blivit förlängd med belysning till 16 timmars dagsljus följt av 8 timmars mörker. Med LED-ljus är det möjligt att designa ljusets färg utefter specifika våglängder, vilket har öppnat upp för en diskussion om den mest fördelaktiga typen av ljus för en mjölkko. Syftet med den här avhandlingen var att undersöka ljusmiljöernas roll och påverkan av ljusintensitet, ljusets våglängdssammansättning och ljusets jämnhet i rummet (uniformitet) för mjölkkor. I de första studierna användes ett ljuslabb med en kontrollerad ljusmiljö och inget externt ljus. Effekten av rött, blått och vitt ljus med ökande intensitet på pupillstorleken utvärderades hos fem dräktiga sinkor. Rött ljus påverkade inte pupillens storlek, medan de andra ljusen gjorde det. Därefter testades 16 timmars blått, rött och vitt ljus på dagen och 8 timmars svagt vitt nattljus på 40 dräktiga, lakterande kor under fem veckor. I studien sågs inte några skillnader i mjölkmängd mellan kor i de olika ljusbehandlingarna. Melatonin i plasma var högre under svagt nattljus än i dagsljus för alla ljusbehandlingar. För att testa kors rörelsemönster i belysningar med olika intensitet, spektral sammansättning och uniformitet undersöktes hur 12 dräktiga, sinkor navigerade genom en hinderbana i ljuslabbet. Att alla lampor var släckta, begränsade inte kornas förmåga att gå genom hinderbanan. Däremot minskade korna ganghastigheten vid ojämnt, svagt rött ljus, vilket talar för att ojämnt ljus bör undvikas i mjölkladugårdar. En delstudie där ljusmiljöer på fyra mjölkgårdar kvantifierades med olika mätmetoder visade på låg ljusintensitet och låg uniformitet i vissa områden på samtliga gårdar. Ljusmiljön är viktig för mjölkkorna eftersom den kan påverka deras fysiologi och beteende. Genom att använda flera mätmetoder för ljus och inte bara sådana som är anpassade efter människans synförmåga, kan ljusmiljön beskrivas mer objektivt för mjölkkor.

Nyckelord: Mjölkproduktion, nattbelysning, svag belysning, arenatest, gångbeteende, pupillrespons, melatonin, IGF-1, hinderbana

Dedication

Till mormor och morfar.

Happiness can be found even in the darkest of times, when one only remembers to turn on the light

Prof Albus Dumbledore, JK Rowling

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Lindkvist S, Ternman E, Ferneborg S, Bånkestad D, Lindqvist J, Ekesten B, Agenäs S (2021). Effects of achromatic and chromatic lights on pupillary response, endocrinology, activity, and milk production in dairy cows. *PLOS ONE* 16 (7), e0253776.

II. Lindkvist S, Ferneborg S, Ståhlberg K, Bånkestad D, Ekesten B, Agenäs S, Ternman E. Impact of light intensity, spectrum and uniformity on ability on cows to navigate through an obstacle course. (Submitted to *Journal of Dairy Science,* March 2023)

III. Lindkvist S, Ekesten B, Agenäs S, Ternman E. Characterization of light on dairy farms (manuscript).

Paper I is reproduced with the permission of the publisher

The contribution of Sofia Lindkvist to the papers included in this thesis was as follows:

I. Coordinated the planning of the animal trial and organized the set-up of the light lab. Had main responsibility for running the trials. Analyzed the results in collaboration with the supervisors, prepared the first draft of the manuscript and adjusted the manuscript after discussions with the co-authors.

II. Planned the animal trial, including choosing and designing light treatments in which to test the cows. Was responsible for conducting the trial. Analyzed the results in collaboration with one supervisor. Prepared the first draft of the manuscript and adjusted the manuscript after discussions with the co-authors.

III. Planned in collaboration with the supervisors. Conducted data collection on farms. Prepared and collated the data and had the main responsibility to summarize the results. Prepared the first draft of the manuscript and finalized the manuscript together with the co-authors.

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Abbreviations

AMS	Automated milking system
DMI	Dry matter intake
ELF	Environmental light field
GHG	Greenhouse gas
IGF-1	Insulin-like growth factor 1
ipRGCs	Intrinsically photosensitive retinal ganglion cells
L-cones	Long wavelength-sensitive
LDPP	Long day photoperiod (16-18 h light; 6-8 h darkness)
LED	Light-emitting diodes
Lx	Lux
ML-cones	Medium to long wavelength-sensitive
NDPP	Natural day photoperiod (9.5 to 14.5 h of daylight)
PFD	Photon flux density
RAP	Relative area of the pupil
S-cones	Short wavelength-sensitive
SCN	Suprachiasmatic nuclei
SDPP	Short day photoperiod (8h light; 16 h darkness)
SRB	Swedish Red Breed

1. Introduction

Artificial light can be used as a management tool in modern dairy production. In the past, lighting in dairy barns was not always prioritized. This is evident in a doctoral thesis examining dairy farm practices in Sweden in the period 1850-1914 (Martiin published as Israelsson, 2005), which cites statements from the first edition of the animal management book *Husdjurslära* by agronomist Hjalmar Nathorst, published in 1859. Nathorst stated that darkness reduces cows' appetite, resulting in lower feed consumption and making feed last longer, and that darkness and limited space are beneficial for the growth rate in young cattle. The book also recommended covering windows and small openings in barns, to avoid low temperatures and cold draughts. This kind of instruction would have restricted the influx of natural light, and the recommendation to keep cows in darkness was changed two decades later, also by Nathorst. In the second edition of *Husdjurslära*, published in 1876, Nathorst focused on light instead of darkness and recommended windows as inlets for natural light, despite the risk of low temperature in the barn. He also referred to positive effects of light in preventing diseases (Martiin published as Israelsson, 2005).

Today, we know that milk production increases if the natural day (9.5 to 14.5 h of daylight) is extended to a long day (16 to 18 h of daylight) by artificial light (Dahl *et al*., 2000). In addition, animal caretakers working in cow barns need adequate light for their safety and work quality. Light for people's work is regulated in the European standard for lighting in workplaces (EN 12464-1, 2021). It has been shown that appropriate light improves staff working conditions (Cajochen, 2007) and can lead to improved care of the animals and improved cleanliness. Both these effects likely contributed to the improvements observed by Nathorst that made him change his mind about light in barns.

Barns built from the mid- to late 1800s exemplify this shift towards brighter indoor environments. One such barn, now housing a restaurant, can be found on the Swedish University of Agricultural Sciences campus at Ultuna, Uppsala. That barn was designed by Charles Emil Löfvenskiöld, who substantially influenced the design of livestock buildings during the middle to the end of the 19th century, and many of his designs had large windows (Svala, 1992).

Artificial light in dairy barns was introduced around the 1920s in Sweden, during electrification (Martiin, 2016). However, interest in the light environments in dairy facilities has varied since then. When it was shown scientifically that a long day photoperiod (LDPP) of 16 to 18 h of daylight per 24 h increases milk production, as first reported by Peters *et al*. (1978) and later by others (Miller *et al*., 1999; Dahl *et al*., 2000), light was established as a key component for successful dairy farming. Today, national animal welfare acts tend to have a section regulating the provision of natural light or the use of artificial light, or both, in dairy barns. Typical concerns on farm level when obtaining a preferred light environment are the cost of installing new light fixtures, practicalities regarding replacing the bulbs or fluorescent lamps when they stop working, fire hazards and, in periods with high electricity costs, the energy requirements of the lights.

When light-emitting diode (LED) lights for animal houses became available they quickly attracted the interest of farmers, since they use less energy and last longer than other light sources. In addition, the possibility of designing LEDs to emit specific light wavelengths triggered a discussion on the most favorable type of light for dairy cows. However, evidence on the impact of light intensity, spectrum and uniformity on dairy cows is still scarce.

2. Background

2.1 Cow vision

Most herbivores have their eyes placed laterally, while predatory species have their eyes set well forward. This gives herbivores a panoramic field with 330° to 360° vision, mainly to protect themselves from predators when grazing (Prince *et al*., 1960). Because of the placement of the eyes, cows only have a small field (52°) of binocular overlap where both eyes focus on the same object, resulting in limited capacity for binocular perception of depth (Hughes, 1977).

Cattle have two major types of photoreceptors involved in vision: rods and cones (Greef, 1894). Like most mammals, cattle are dichromats and have short wavelength-sensitive cones (S-cones) and medium to long wavelength-sensitive cones (ML-cones), with peak sensitivities at 451 nm (blue) and 555 nm (greenish-yellow), respectively (Jacobs *et al*., 1998). Bovine rods are most sensitive to 498 nm (bluish-green) (Partridge & De Grip, 1991; Hofmann & Lamb, 2023). Although the exact proportions of cones and rods in the bovine retina remain unknown, cows have been shown to have a rod-dominated retina (Schiviz *et al*., 2008).

The eye also provides sensory input for non-image-forming visual functions, including circadian photoentrainment for setting internal biological clocks, inhibition of melatonin release (which plays a pivotal role in the sleep-wake cycle) and adjustment of the number of photons reaching the retina through the pupillary light reflex (Campbell & Gregory, 1960; Hattar, 2002; Altimus *et al*., 2008; Reifler *et al*., 2015). A third group of photosensitive receptors in the retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the photopigment melanopsin, drive or contribute to regulation of all these functions (Provencio *et al*., 1998; Hattar, 2002; Lucas, 2003; Dacey *et al*., 2005; Qiu *et al*., 2005).

2.1.1 Photoreceptors

Rod and cone photoreceptors have different functions (Schultze, 1866), where rods mediate perception in dim light and cones are specialized for brighter light and color vision. When adapting to a dark environment from a bright light environment, there is a shift from activation of cone pigments to very light-sensitive rhodopsin, together with pupil dilation, to enable proper dim light vision (Walls, 1942). For example, vision is limited when entering a dark environment due to the low amount of rhodopsin in rods. At the same time, the pupil dilates to allow more photons to reach the retina. In humans, pupil dilation is almost completed within one minute and fully completed in 10 minutes at low light levels (Wagman & Gullberg, 1942). The opposite response, pupil constriction, is a rapid process and retinal cone adaption seems to be completed in less than 10 minutes in humans (Asakawa *et al*., 2019).

For a long time, constriction of the pupil in daylight was considered to be driven by retinal cones and mainly related to light intensity. More recently, it has been postulated that while photoreceptors play a role in regulating pupil size, steady-state pupil size is mainly controlled by ipRGCs (Gamlin *et al*., 2007; McDougal & Gamlin, 2010). The expression 'steady-state pupil' refers to when the size of the pupil is held steady under continuous light. The ipRGCs also receive input from retinal cones and rods (Dacey *et al*., 2005; Weng *et al*., 2013), although the cone input continues for less than a minute to pupillary constriction when constant levels of light are used. Rods may contribute longer, but only at light levels below saturation of the rod response (McDougal & Gamlin, 2010).

Red light is a relatively weak stimulus for rod photoreceptors, as the wavelengths perceived as red (600-700 nm) are far from the peak sensitivity of rhodopsin (498 nm). Hence, dim red light is often used when maintaining a relatively dark-adapted state of the retina is required, *e.g*. in photography darkrooms. With brighter red lighting, the retina becomes more light-adapted and the number of photons starts to activate long wavelength-sensitive cones (L-cones) or ML-cones under mesopic (both rods and cones are active) light conditions (Pokorny *et al*., 2006). Bright red light eventually saturates the rod photoreceptors under photopic light conditions, and vision is then mediated through the cone photoreceptors (Ofri & Ekesten, 2021).

2.1.2 Color perception

Bovine color perception has been investigated throughout history and many knows the saying 'like a red rag to a bull'. However, a study by Stratton (1923), based on a survey of cattle breeders, on whether the color red excited bulls more than other colors found no evidence that red is more arousing for cattle. A more recent study by Dabrowska *et al*. (1981) using colored cards to investigate color perception in adult cows found that they could differentiate red from several different shades of grey.

Studies have also examined whether cattle can distinguish red light (600-700 nm) from lights containing other wavelengths (Gilbert & Arave, 1986; Phillips & Lomas, 2001). These studies investigated whether heifers can differentiate between red (610 nm), green (535 nm) and blue (450 nm) colors (Gilbert & Arave 1986), and whether calves can distinguish between red, green and blue light with peak wavelength 635, 525 and 415 nm respectively (Phillips & Lomas, 2001). Both studies found that the animals could distinguish red light from green and blue light. Phillips and Lomas (2001) also tested the effect of light color on calf behavior and found a higher number of movements per minute in red light than in green or blue light.

2.2 Circadian rhythms

Circadian rhythms in mammals are controlled by their internal biological clock and correspond to the solar day, which on Earth averages 24 hours (Freedman *et al*., 1999). The biological clock is located in the suprachiasmatic nuclei (SCN) of the hypothalamus. Light provides sensory input that inhibits melatonin release, which plays a pivotal role in the sleep-awake cycle (Hattar, 2002; Bradshaw & Holzapfel, 2007; Altimus *et al*., 2008), together with other factors. The ipRGCs contain the photopigment melanopsin, which drives or contributes to melatonin release and pupillary light reflex (Provencio *et al*., 1998; Hattar, 2002; Lucas, 2003; Dacey *et al*., 2005; Qiu *et al*., 2005).

In humans, the most potent part of the spectrum providing circadian input for regulating melatonin secretion is around 446-477 nm (Lockley *et al*., 2003; Dacey *et al*., 2005; Brainard *et al*., 2008). These wavelengths are close to the peak absorption of murine melanopsin (479 nm) (Lucas *et al*., 2001), but also bovine S-cones (451 nm) (Jacobs *et al*. 1998).

The diurnal rhythm, or pattern, comprises a specific sequence of daytime and night-time activities. Dairy cows show diurnal rhythms both on pasture and when housed, even though their indoor environment differs significantly from the outdoor environment in which their ancestors lived (Kilgour, 2012). On pasture, the main activities of cows are grazing, ruminating and resting, with resting activity dominating during the night and grazing during the day (reviewed by Kilgour, 2012). Cows housed indoors maintain a similar diurnal rhythm as cattle on pasture (Munksgaard *et al*., 2011). In addition, cattle tend to display crepuscular feeding behavior, *i.e.* with peak feed intake during dawn and dusk (Ray & Roubicek, 1971; Ruckebusch & Bueno, 1978).

2.3 Features of light

The indoor light environment in dairy barns depends on several factors, the most obvious being artificial light fixtures. Depending on the light fixture type and placement, the light environment can be experienced differently. Daylight inlets and the building design also affect the indoor light environment, *e.g*. daylight inlets by the roof ridge can allow sunlight into the interior of the building. Small windows or large openings can be placed along the sides of the building, with the size of these determining the amount of natural light indoors. An additional factor that is often overlooked when designing indoor lighting in dairy barns is the texture and color of interior surfaces. Black surfaces have lower reflectance than white, while surface properties on the matt to shiny scale differ in reflectance (Gilchrist, 1979). In addition, the geographical location of the building, its orientation and the season of the year affect the available outdoor light, and hence indoor light.

The outdoor light environment differs greatly from the indoor environment in animal houses. For example, the visual outdoor light environment for animals on pasture can be described as a bright sky with a dark shift at the horizon and green ‘floor’ (Nilsson *et al*., 2022). This is in stark contrast to the visual indoor light environment in Swedish dairy barns, where the roof ridge is the brightest, there is a dark ceiling and a bright horizon because of the windows, and the floor is dark. Another difference is that outdoor light intensity varies during the solar day and is also affected by weather, the lunar calendar and light pollution from the moon, airglow or nearby city lights (Cinzano *et al*., 2000; Falchi *et al*., 2016). In order to learn about the effects of different light sources on animal behavior, it is necessary to measure (quantify) light accurately.

2.3.1 Light characterization

Light intensity can be quantified by numerous methods, which can be subdivided into three different categories: radiometry, photometry and photonmetry (Fujiwara, 2016). Radiometry describes the energy basis, e.g. irradiance (Wm^{-2}) is an example of a radiometric quantity. Photometry determines the amount of light illuminating a surface, i.e. the illuminance, which is wavelength-weighted based on human photopic vision and is measured in lux (or lumens/m^2). Other photometric quantities are luminous flux (lumens) and luminance (candela/m^2). Radiometry and photometry are well known techniques, and photometry is often used when designing light in buildings for humans and livestock (Sliney, 2016). However, the measurement of illuminance in lux is based on the properties of the human eye and vision. A lux meter has peak sensitivity at 555 nm (Figure 1; green light), which fits well with the human peak sensitivity under photopic conditions (Schnapf *et al*., 1987; Sharpe *et al*., 2005).

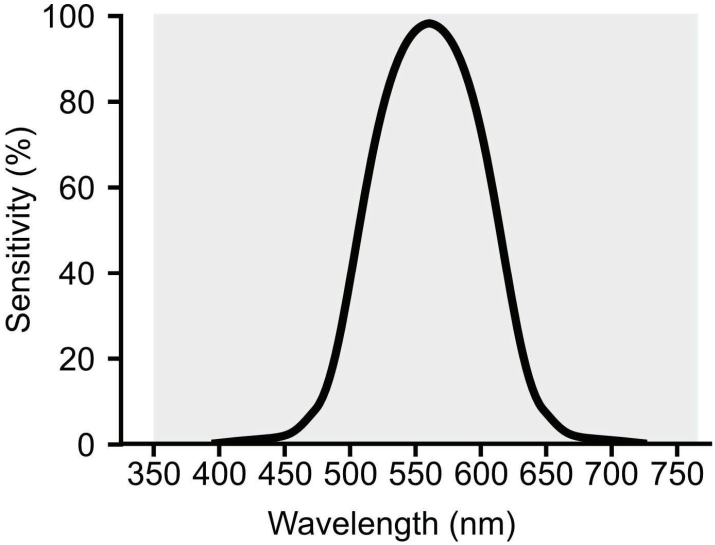

Figure 1. Illustration of peak sensitivity of a lux meter (black curve). The grey area indicates the sensitivity area of a spectrophotometer within the field of visible light.

Photonmetry is an emerging technique that has been used to describe plant responses to light and characterize the photon flux (flow rate). To describe the photon-based light intensity, photon flux density (PFD; µmol m^{-2} s^{-1}) can be used (Fujiwara, 2016). A spectrophotometer that measures PFD per wavelength (µmol m^{-2} s^{-1} nm^{-1}) characterizes the available light within a specified spectrum. This could be the spectrum of visible light (350 nm to 780 nm), while for some animals perceiving shorter wavelengths it may also be relevant to include UVA light (315-400 nm) (Douglas & Jeffery, 2014). From the spectrophotometer, it is possible to get information about the total number of photons available and also the number of photons per wavelength. With the information per wavelength, it is possible to assess the spectral distribution of the light. Within the spectrum of visible light, shorter wavelengths (350-500 nm) are perceived as violet to blue to cyan by a human with normal color vision, medium wavelengths (500-600 nm) are perceived as green to yellow to orange, and long wavelengths (600-700 nm) appear red (Figure 2). The light that humans perceive as white light often contains a mixture of the different wavelengths.

Another method for characterizing the light environment is image processing (Nilsson & Smolka, 2021). The environmental field method (ELF) uses image analysis to quantify biological aspects of light environments, based on photographs of the environment taken using a digital camera with a 180° fisheye lens. The results provide graphical information about the light environment and about the light intensity measured as $\log_{10}$ number of photons s^{-1} m^{-2} sr^{-1} nm^{-1} (lit).

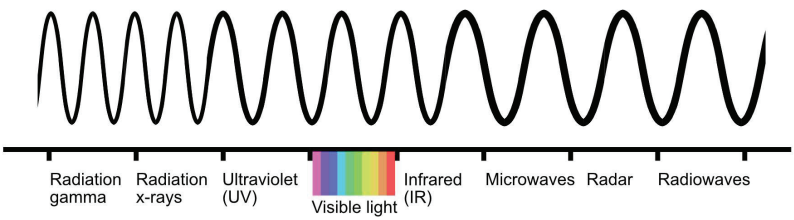

Figure 2. The electromagnetic spectrum from gamma radiation to radiowaves, including the spectrum of visible light between 350 nm to 780 nm.

2.3.2 Uniformity

When designing light environments, uniformity of light is often mentioned with reference to how evenly light is distributed over a specific area. If uniformity is high, light measurements will not differ at different positions within the area. If uniformity is low, some positions in the area will have higher light intensity and some lower. Light uniformity is often discussed in a traffic safety and road lighting context, and also with regard to working environments for humans. According to the European standard for lighting in workplaces, the light environment should have high uniformity (≥0.7) (EN 12464-1, 2021). Uniformity is calculated by dividing the minimum light intensity by the average light intensity, with values below 0.7 considered to indicate low uniformity. For night-time lighting in dairy cattle facilities, it is common to leave a few light fixtures on or to use supplementary lighting, resulting in low light uniformity.

2.4 Light for dairy cows

Previous research on the effect of light on dairy cows has revealed several important factors. The benefits of LDPP during an ongoing lactation on milk production have been well demonstrated (Dahl *et al*., 2000). Circulating melatonin shows a diurnal pattern in cows when exposed to a light and a dark phase within 24 h, with low levels of melatonin during exposure to light and increased levels during exposure to darkness (Hedlund *et al*., 1977; Muthuramalingam *et al*., 2006; Bal *et al*., 2008; Kollmann *et al*., 2008; Elsabagh *et al*., 2020). In addition, effects of light color on melatonin release have been reported in dairy calves, with blue LEDs suppressing the expected melatonin increase in the evening compared with yellow LED light (Elsabagh *et al*., 2020).

It has also been shown that LDPP increases circulating insulin-like growth factor-1 (IGF-1) in heifers (Spicer *et al*., 2007) and in lactating cows (Dahl *et al*., 1997). However, whether IGF-1 is a supporting factor for maintaining an ongoing lactation is a moot question (Dahl *et al*., 2000). A correlation between melatonin and IGF-1 has been reported, *e.g*. a negative (although non-significant) correlation during night-time in dairy heifers (Muthuramalingam *et al*., 2006).

Light can also affect cow behavior. As mentioned, cows show a diurnal pattern when housed, and some cow behaviors are thought to be connected to the availability or the lack of light. A study with 24-h light found that cows still spent less time eating and more time lying down during the night hours (Munksgaard *et al*., 2011), indicating that factors other than light affect diurnal behaviors. However, those authors do not state whether the cows had an acclimatization period before data collection. If they were not allowed to acclimatize, they might have kept their diurnal behavior from before measurements began. Another study found that the resting time of cows was greater during periods of darkness than when exposed to light, even when the dark period was phase-shifted (Suarez-Trujillo *et al*., 2020).

Studies on cow movements in darkness have found contradictory results. For example, a study comparing gate passages under three different night light intensities (11±3, 33±1 and 74±6 lx) on automated milking system (AMS) farms found that the number of gate passages per 24-h period did not differ between the light intensities (Hjalmarsson *et al*., 2014). However, regardless of night-time light intensity, the cows passed through the gates more frequently during daytime than at night (Hjalmarsson *et al*., 2014). In another study where cows could choose a bright or a dark passageway, almost all cows avoided the dark passageway (Phillips & Morris, 2001). Moreover, in a study comparing cow locomotion in a passageway with different light intensities, Phillips *et al.* (2000) found that stride rate was higher and stride length shorter in dim light than in bright light, but that walking speed (m/s) was similar in both light environments. In addition, it has been shown that daylight inlets and a bright sunny day can create shadows indoors that affect walking behavior and hinder cow traffic (Willson *et al*., 2021).

The light environment can improve milk production and produce more milk per kg feed. Feed production is a significant source of greenhouse gas (GHG) emissions from dairy farms, comprising around 85% of the total milk carbon footprint (Flysjö *et al*., 2011). Milk carbon footprint varies between dairy farms, due to management differences (Henriksson *et al*., 2011), with a higher ratio of milk to feed leading to lower GHG emissions, indicating scope for improvement.

In addition, LEDs have lower energy consumption than older light fixtures and have a longer lifespan, reducing farm costs and the environmental load from electricity use. Around 10% of the electricity used in dairy barns is reported to pertain to light sources, although those measurements were primarily performed in 2005-2006 when LEDs were not in widespread use on farms (Hörndahl & Neuman, 2012).

Most studies on light programs or environments for dairy cattle, report the light source used, photoperiod length, group of animals (*e.g.* age and category), days in lactation, and sometimes lux measured at a specific height. However, the type of measuring instrument used and exactly how the measurements were performed are seldom described. This lack of information about the light environments used in different studies limits the possibility to replicate studies and to critically analyze the results.

3. Aims

The main aim of this thesis was to determine the role of light environments and the impact of light intensity, spectrum and uniformity on dairy cows.

Specific objectives were to:

- Develop a protocol for light characterization pertinent to dairy cow biology (**Papers I-III**)
- Study the effects of light of differing spectral composition on activity, milk production, endocrinology and pupillary response in dairy cows (**Paper I)**
- Evaluate whether low and non-uniform light intensities limit the ability of cows to navigate indoors (**Paper II**)

4. Material and methods

The material and methods employed in the different studies on which this thesis is based are summarized below, while detailed descriptions can be found in Papers I-III. The work reported in Papers I and II was conducted at the Swedish Livestock Research Centre in Uppsala, Sweden. Collection of data for **Paper III** was conducted on dairy farms in Sweden. All three studies were carried out during the period 2019-2023. The Uppsala Ethics Committee for Animal Research approved the experimental design and all handling of animals (reference no. 5.2.18-11064/16 and 5.8.19-06780/2020 for **Paper I** and **Paper II**, respectively).

4.1 Light measurements

Characterization of light in the different studies was performed using several different instruments. All light measurements were performed at cow eye level, approximately 1.3 m above the floor, with the instruments attached to a tripod to ensure stability, exact height and free sensor sight directed upwards. Illuminance (lux), luminance (candela/m^2), PFD (µmol m^{-2} s^{-1}), light spectrum (µmol m^{-2} s^{-1} nm^{-1}) and spectral photon radiance (lit; log_{10}photons m^{-2} s^{-1} sr^{-1} nm^{-1}) were quantified. The instruments used in Papers I-III are specified in Table 1.

Table 1. Physical entities and instruments used to characterize and quantify light environments in Papers I-III in terms of: illuminance (lx), luminance (candela/m^2), photon flux density (PFD; µmol m^{-2} s^{-1}), light spectrum (µmol m^{-2} s^{-1} nm^{-1}) and spectral photon radiance (lit; log_{10}photons m^{-2} s^{-1} sr^{-1} nm^{-1})

	Illuminance	**Luminance**	**Photon flux density**	**Light spectrum**	**Spectral photon radiance**
Paper I	x[1]	x[3]	x[4]	x[4]	
Paper II	x[1,2]		x[2,4]	x[2,4]	x[5]
Paper III	x[1]		x[2,4]	x[2,4]	x[5]

[1]Hagner Screenmaster, B. Hagner AB, Solna, Sweden.

[2]PAR200, Quantum Spectrophotometer, UPRTEK, Europe, Aachen, Germany.

[3]IL-1700, International Lights, Peabody, MA, USA.

[4]Jaz, Ocean Insight, Inc. Dunedin, Florida, USA.

[5]Environmental light field (Nilsson & Smolka, 2021).

4.1.1 Spectral distribution

In **Paper I**, three different colors of light were used: blue, red and white (see Figure 3A, 3C, 3D for spectral distribution). The white light (Figure 3A) was a mixture of blue, green and red light, with peaks at 425 nm and 660 nm, aiming for similar amounts of blue and red colored light in the mixture. In **Paper II**, white and red colors were used (Figure 3B, 3D), aiming for similar amounts of blue, red and green colored light in the mixture.

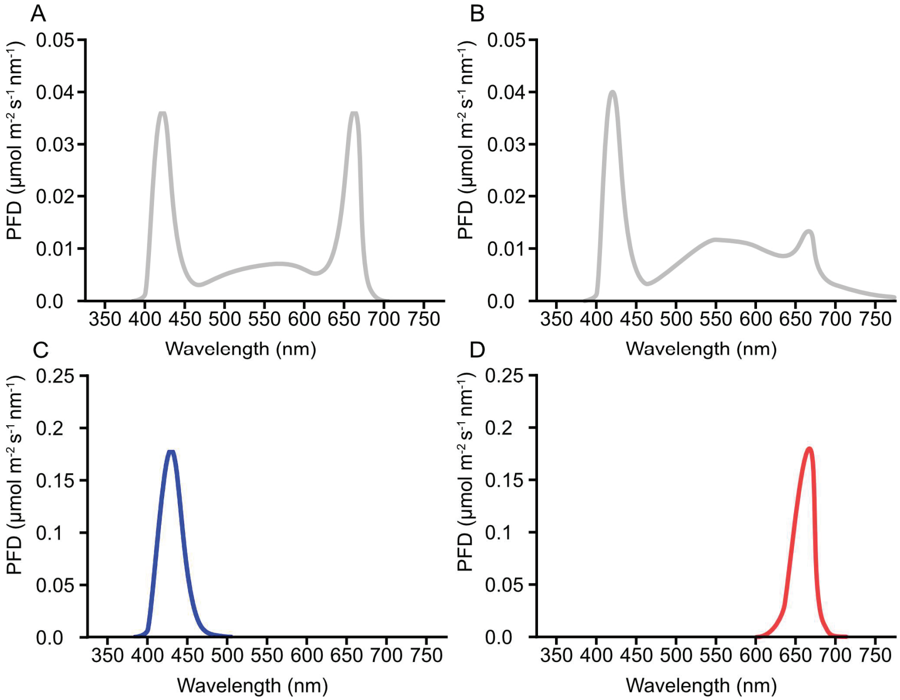

Figure 3. Spectral distribution of the light treatments used in **Paper I** (A,C,D) and in **Paper II** (B,D). A and B are perceived as white light by a human trichromat, C as blue light and D as red light.

4.2 Paper I

In **Paper I**, two studies were performed to investigate the effects of differing spectral composition of artificial light on pupillary response, endocrinology, activity and milk production in lactating dairy cows. Both studies were carried out in a tie-stall barn without any external light, enabling provision of a controlled light environment (light lab). The light lab had two rows of tie-stalls, each row facing a wall, and one light treatment could be applied per row, allowing two light treatments to be tested at the same time. Light was provided by LED light fixtures (Elixia LX602G, Heliospectra AB, Sweden) placed on each side of the head of every cow, approximately 140 cm above the forehead of a cow standing up. The LEDs in the light fixtures were remotely controlled and both intensity and the spectral composition of the light could be adjusted.

4.2.1 Study A

The starting hypothesis in Study A in **Paper I** was that pupil response to light is driven by photon flux and does not differ between different wavelengths. This hypothesis was tested by applying blue, red and white light of increasing intensity in the light lab and assessing the response in terms of size of the pupils in five pregnant non-lactating cows of the Swedish Red Breed (SRB). The dimmest light was applied first and the intensity was then increased step-wise, allowing the cows to adapt to each new light environment for 10 minutes before taking a photograph of each eye at approximately 2-3 m distance.

Relative area of pupil (RAP) was calculated as the area covered by the pupil in the photograph divided by the area circumscribed by the peripheral iris at the limbus cornea (Figure 4). To estimate the amount of light that reached the retina, and to enhance comparison with conventional retinal illumination measured in Trolands (equal to pupil area in mm^2 times luminance in candela/m^2) (Thibos *et al.*, 2018), the photon flux was multiplied by mean RAP.

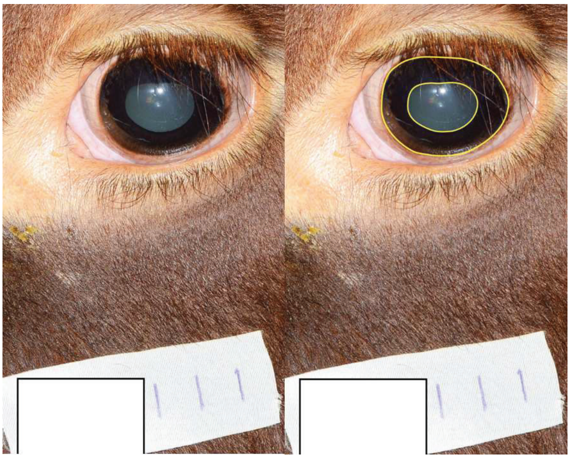

Figure 4. Photographic illustration of calculation of relative area of pupil (RAP) in dairy cows. The yellow rings in the right-hand photograph delineate pupil area and iris area.

4.2.2 Study B

The starting hypothesis in Study B in **Paper I** was that blue light during the day increases cow activity at night and that red light does not support the diurnal release pattern of melatonin as well as blue or white light. To test this hypothesis, 40 lactating SRB cows in two blocks (n=20 per block) were studied in the light lab. A long-day photoperiod (LDPP) was used, with 16 h of daylight and 8 h of dim night light.

During period 1, the light environment in daytime was of blue color (34.7 µmol m^{-2} s^{-1}) for one group (n=10) and of red color (34.9 µmol m^{-2} s^{-1}) for the other group (Figure 5). During period 2, the light environment in daytime was of white light (36.9 µmol m^{-2} s^{-1}) for one group, while the other group had the same white light for 10 h and then switched to blue-colored light (34.7 µmol m^{-2} s^{-1}) for the last six hours of the daytime period. The four different daylight treatments had the same dim white night light with intensity 0.18 µmol m^{-2} s^{-1}.

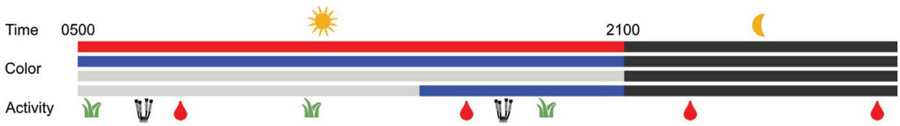

Figure 5. Schematic illustration of the light treatments per 24-hour period used in **Paper I**. Daylight was provided for 16 hours and dim night light for eight hours. Symbols indicate times of feeding ( ), milking ( ) and blood sampling ( ).

The cows spent 33 days in each light treatment. Feed intake, milk yield and composition, and standing and lying activities were recorded during the last five days of the treatment period. During the last 24 hours of the treatment period, blood was sampled four times (at 08.30, 16.00, 22.30 and 04.00 h) and analyzed with an ELISA kit for melatonin and IGF-1.

4.3 Paper II

Paper II investigated the ability of dairy cows to navigate in different indoor light environments, including light of high and low uniformity, and in two different spectral compositions, red or white light. The study was performed in an indoor test arena containing an obstacle course in a controlled light environment without external light contamination. The test arena was 3.8 m wide and 14.5 m long, with dark rubber mats covering the floor, and had 18 seamlessly dimmable LED light fixtures lighting the obstacle course. These light fixtures were placed in two rows at 2.9 m above the floor and were connected to a computer that controlled the intensity and spectral composition of the light provided by each light fixture (Figure 6).

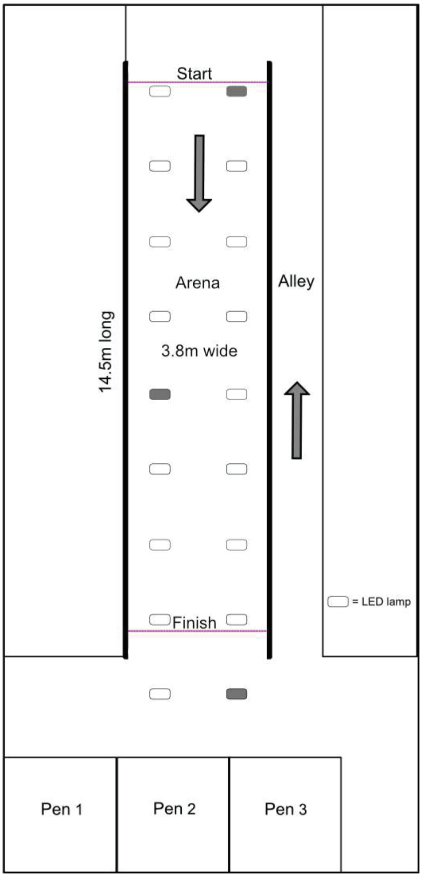

Figure 6. Illustration of the test arena used in **Paper II**. Light was supplied by 18 LED lamps, and filled rectangles (grey) indicates lamps used in non-uniform light treatments.

Twelve pregnant non-lactating dairy cows of the Swedish Holstein breed (n=3) and SRB (n=9) were used in a change-over design, with four batches of three cows in each. One day of acclimatization and training was followed by four test days. Five obstacle courses were tested per cow and day, giving 21 different obstacle courses per cow and batch. The obstacles were white cavalletti poles and plastic cavalletti blocks commonly used in horse training.

Fourteen different light treatments were applied, grouped into two main light regimes: one with uniform light and one with non-uniform light. The treatments were further subdivided into color of light (white and red) with five light levels (dark, low_1, low_2, medium and high). All 18 light fixtures were used for the uniform light scheme, while the non-uniform light was achieved by using only three fixtures (Figure 7).

Through direct observations, the number of strides taken through the obstacle course and the time cows spent in the test arena were noted. Other behaviors were indirectly mapped from video recordings, and cow heart rate and respiratory rate were measured.

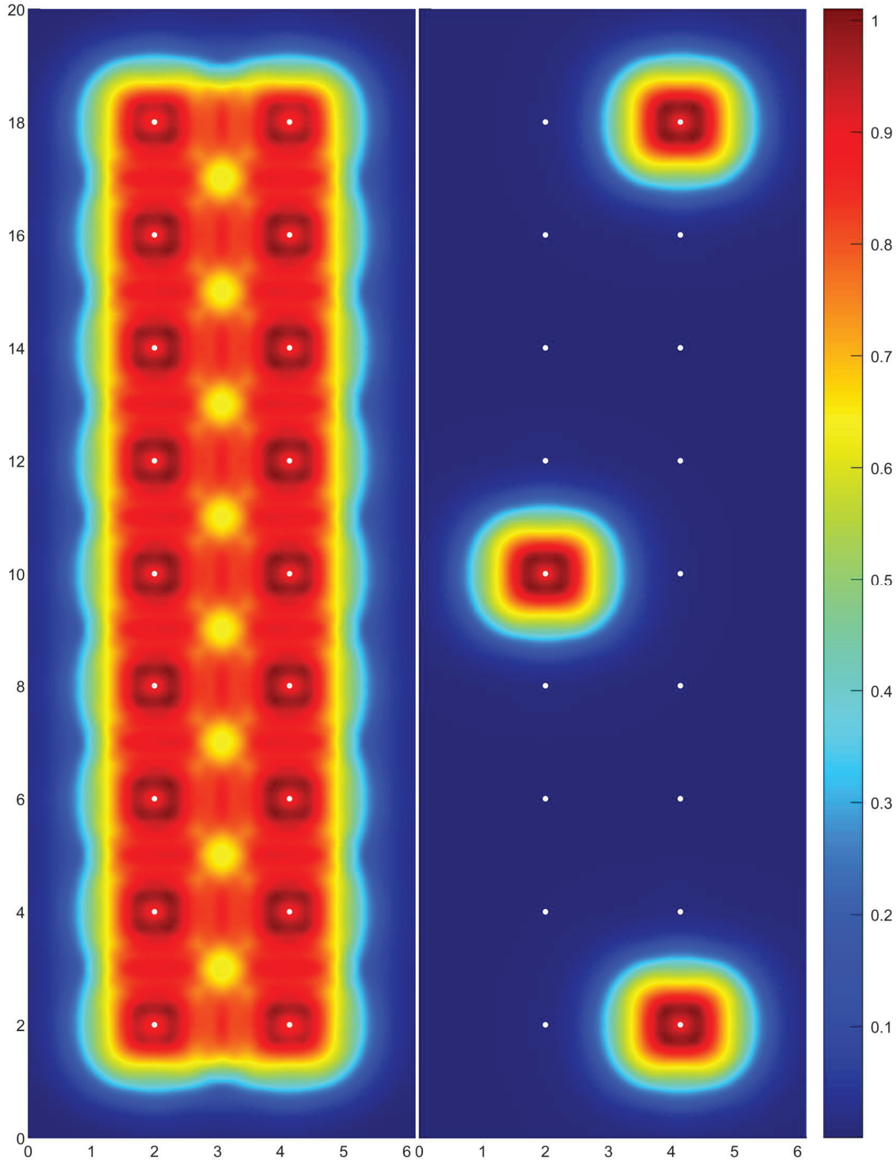

Figure 7. Heat maps of the light environment in the test arena used in **Paper II** showing light uniformity when (A) all lamps were lit, corresponding to a high light uniformity treatment, and (B) when only three lamps were lit, corresponding to a low light uniformity treatment.

4.4 Paper III

Paper III examined variations in light conditions in dairy barns in Sweden and sought to develop a method for light measurements in dairy barn environments. Four dairy farms in Sweden (Farms A-D) were included in the study and light was measured in two different settings. All measurements were made after sunset, ensuring that artificial light was measured, with no impact of outdoor light. The two different settings were as follows: Farm A and Farm B changed their light fixtures from fluorescent to LED lights, and measurements were made before and after the change. On Farm C, measurements were performed in both the daytime and the night-time light regime, while on Farm D light measurements were made in two different areas in the barn during the daytime light regime. Before every measurement, the outline of each building, its interior colors and daylight inlets were studied in order to plan the procedure. There were differences in the measurement tools used on the different farms (Table 2). From the measured values, mean, minimum and maximum intensity were analyzed. Light uniformity was calculated as the minimum light intensity divided by the average light intensity, according to the European standard for lighting in workplaces (EN 12464-1 2021).

Table 2. Light characterization procedure on four dairy farms in Sweden (Farms A-D) using different techniques.

	No. of lactating cows	**Illuminance**	**Photon flux density**	**Light spectrum**	**Spectral photon radiance**
Farm A	350	x	x	x	x
Farm B	450	x			x
Farm C	65	x	x	x	
Farm D	290	x			x

4.5 Data handling and statistical analysis

All data in Papers I and II were checked for normality and outliers using the univariate procedure in SAS (version 9.4, SAS Institute Inc., Cary, NC., USA). Where applicable, data were $\log_{10}$-transformed.

Unless otherwise stated, the values presented are least squares mean (LSM) ± SEM. Results were considered significant at $P \leq 0.05$, while a trend was assumed for probabilities $0.10 > P > 0.05$. Post-hoc means separation for significant main effects was applied using Tukey-Kramer's adjustment of probability values.

4.5.1 Paper I

A generalized mixed model in SAS was used to test whether pupil size was affected by light color or intensity. Color, intensity and their two-way interaction were included as fixed effects, and cow nested within treatment as a random effect with an unstructured covariance structure. A generalized mixed model in SAS was also used to assess whether standing, lying, milk production, feed intake, melatonin and IGF-1 were affected by light treatment. In all models, treatment and period were included as fixed effects, and cow nested within treatment as a random effect with an unstructured covariance structure. The model for standing and lying also included the fixed effect of time of day; the model for milk yield, milk composition and feed intake included the fixed effect of days in milk; and the model for melatonin and IGF-1 included the fixed effect of sampling time. Melatonin and IGF-1 were also tested for correlation within 24 hours and at the four different sampling times, using the correlation procedure in SAS.

4.5.2 Paper II

A generalized mixed model in SAS was used to test whether time through the obstacle course (s), number of strides, stride length (m), stride rate (stride/s) and speed (m/s) were affected by light treatment. None of the variables was normally distributed, and all were therefore transformed. All models included light treatment and batch as fixed effects, and cow nested within treatment and batch as a repeated effect, with a first-order autoregressive covariate structure. In addition, the effects of test day, time of day and obstacle course were tested. Finally, both mean and standard error of the mean (SEM) were back-transformed using the delta method (Onofri *et al*., 2010).

After reviewing the occurrence of all behaviors, it was decided to analyze the occurrence ratio only for behaviors that all cows performed in all light treatments (*i.e*. standing still, interaction with an obstacle, interaction with surroundings and interaction with the floor). The odds ratio of a cow performing one of these four behaviors in a light treatment was estimated with a generalized linear mixed model with a binary data distribution, using Proc Glimmix in SAS. Light treatment and batch were included as fixed effects, and cow nested within treatment and batch as a random effect. The proportion of behaviors occurring in each treatment was descriptively illustrated by adding together the number of events per light treatment and dividing by the total number of behaviors occurring.

Differences in physiological data were analyzed using the mixed procedure in SAS. All models included light treatment and batch as fixed effects, and cow nested within treatment and batch as a repeated effect, with a first-order autoregressive covariate structure.

5. Main findings

5.1 Paper I

5.1.1 Study I

Red light of increasing intensity did not constrict the pupil in any of the cows studied, despite an almost 100-fold increase in photon flux. In contrast, the brightest blue and white lights stimulated significant constriction of the pupils at 23.0-23.1 µmol m^{-2} s^{-1} intensity (p<0.001), while already at 1.4-1.5 µmol m^{-2} s^{-1} intensity there was a numerical change in pupil size (Figure 8).

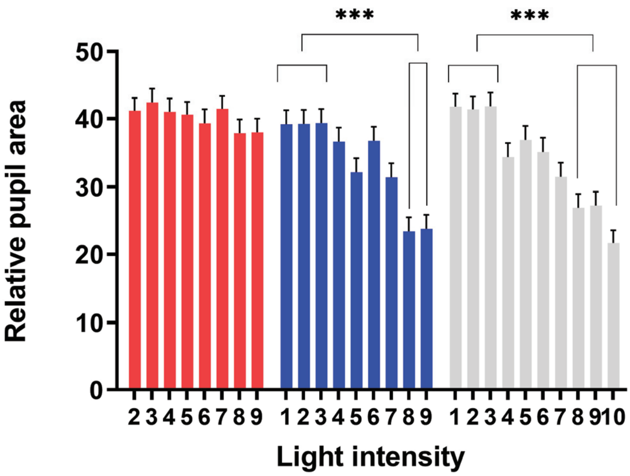

Figure 8. Relative area of pupil (RAP) of dairy cows exposed to (left) red, (center) blue and (right) white light of increasing intensity (***p<0.001). 1.4-1.5 µmol m^{-2} s^{-1} is light intensity number 4 and 23.0-23.1 µmol m^{-2} s^{-1} is light intensity number 8, in blue and white lights, respectively.

5.1.2 Study II

There was no effect of light spectrum on cow activity, dry matter intake (DMI), energy balance or milk composition. Regardless of daytime light treatment, cows spent a higher proportion of the time lying at night than they did during the day. Milk yield was maintained during the five-week study period, with no difference between light treatments. This was an interesting finding, since the cows were in post-peak lactation when milk yield is expected to decrease.

For all light colors, plasma melatonin was higher during dim night light than during daylight ($P<0.001$), with the highest levels of melatonin at 04.00 h in all treatments (Figure 9). In blue and red daylight, the increase in melatonin after switching to dim night light was significantly higher than with white-blue light, and tended to be higher also for the cows exposed to white light. During daytime, there was no difference in plasma melatonin between the light treatments. For all treatments, IGF-1 concentration was lowest at 08.30 h and highest at 22.30 h. No correlation was observed between melatonin and IGF-1 concentration over the 24-hour day-night period, either when samples collected at the same time were compared or when IGF-1 values were compared with melatonin values at sampling six hours earlier.

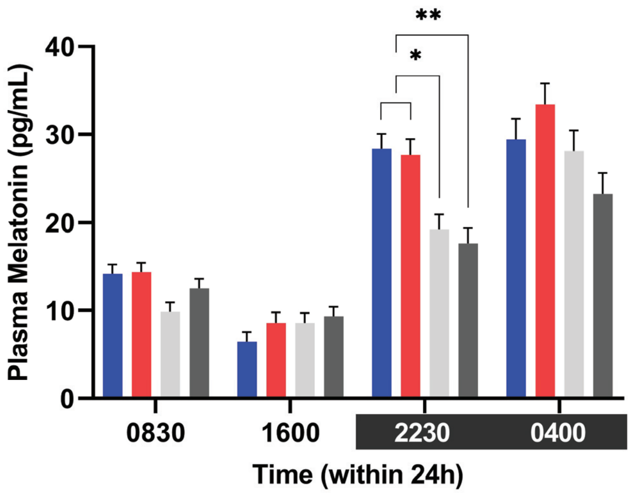

Figure 9. Least square mean (LSM) concentration of plasma melatonin over 24 hours in dairy cows exposed to blue, red, white or white+blue light treatments (blue, red, light grey and dark grey bars, respectively).

5.2 Paper II

There were significant effects of light treatment on cow walking speed (m/s; P=0.006), stride rate (stride/s; P=0.014) and time taken through the test arena (s; P=0.006). Interestingly, pair-wise post-hoc comparisons showed that a dark environment, without any supplementary light, did not alter walking speed or stride rate. Instead, the cows seemed to be more challenged in non-uniform red light, as they spent a longer time in the test arena and walked more slowly in that treatment (Figure 10). The intensity of the non-uniform red light was 0.01-0.46 µmol m^{-2} s^{-1}, compared with 3.19-4.48 µmol m^{-2} s^{-1} for uniform white light or 0.23-0.38 µmol m^{-2} s^{-1} for uniform red light. Cows walked fastest in low-intensity red light (0.28 µmol m^{-2} s^{-1}) with high uniformity. The odds of different behaviors occurring were not affected by light treatment, obstacle course or batch number (Figure 11). Cow heart rate did not differ between the light treatments, but there was a tendency for light treatment to affect respiratory rate (P=0.07).

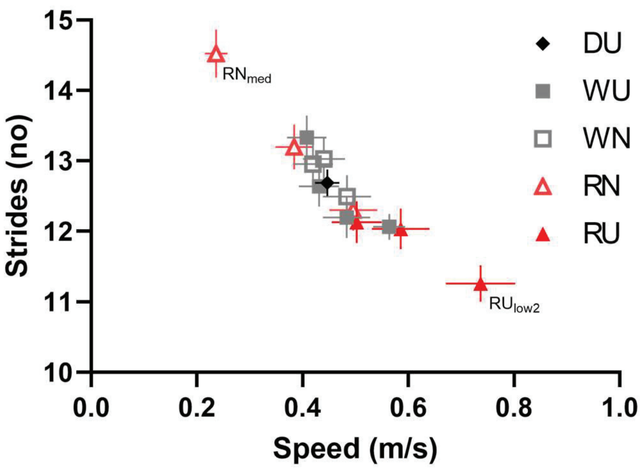

Figure 10. Walking speed (m/s) relative to the number of strides in different light treatments: darkness (D), and white (W) or red (R) light of a uniform (U) or non-uniform (N) nature. Error bars show SEM for walking speed and the number of strides, respectively.

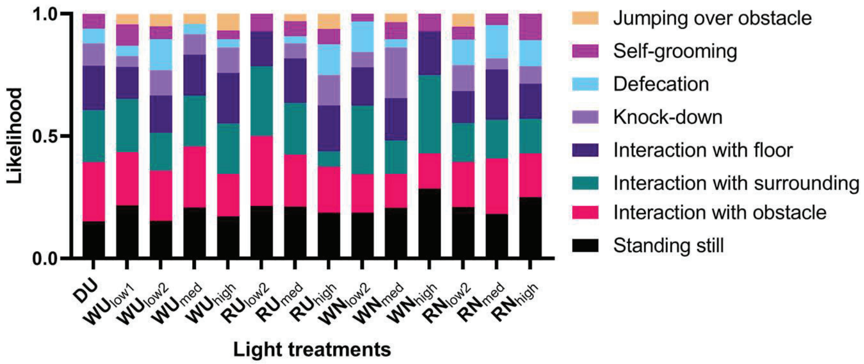

Figure 11. Likelihood of different behaviors occurring in the 14 different light treatments in the test arena, darkness (D), and white (W) or red (R) light of a uniform (U) or non-uniform (N) nature.

5.3 Paper III

On the two farms where the light fixtures were changed from fluorescent to LED lights (Farm A and Farm B), light intensity and uniformity in the free-stall area increased. Specific measurements at the feed table on Farm A and Farm D showed great variation in light intensity between different measuring points. On Farm A, light uniformity at the feed table was 0.24 and light intensity ranged between 55 to 462 lux. On Farm D, light uniformity at the feed table was high (0.69) but light intensity was low (27 to 54 lux). Light environments on the two farms with fluorescent light (Farm C and Farm D) showed similar uniformity (0.22 and 0.19, respectively), independent of light intensity. The night light on Farm C had intensity of 5 ± 3.2 lx and uniformity of 0.20.

Spectrophotometers were used as a complement to light intensity measurements in **Paper III**, since absolute photon flux and spectral composition provide information on how much light is emitted for each color. The results showed that the wavelength spectrum for Farm C comprised mostly green light (500-600 nm), followed by blue (400-500 nm) and red (600-700 nm) light.

According to ELF analysis on Farms A and D (Figure 12), the light environment on Farm A when using LED lights appeared brighter than "mid dusk", comprising mostly red light, and the light environment on Farm D was slightly dimmer than "mid dusk". On Farm A the ceiling appeared slightly dimmer than the floor, whereas the opposite was seen on Farm D, even though they used the same bright-colored bedding material.

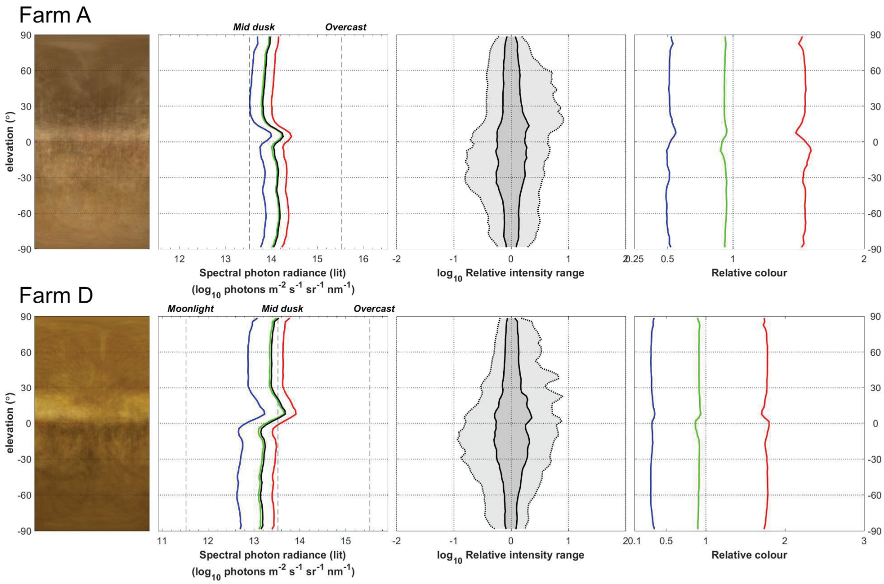

Figure 12. Results of environmental light field (ELF) analysis of the light environment on Farm A and Farm D. The analysis was based on 180° high dynamic range (HDR) images taken within the light lab, with multiple exposures of 25 photos per light treatment taken from different environmental positions, following the cows' progress within the obstacle course. An average image (compressed in azimuth) from the contributing scenes (180° by 180°) is shown to the left, followed by panels showing the intensity (radiance) on an absolute log scale, the intensity range on a relative log scale (dark grey, 50% of all intensities; light grey, 95% of all intensities) and, on the right, the contribution of red, green and blue light plotted on a relative log scale.

6. General discussion

6.1 Light affects cow physiology

Regardless of intensity, red light did not affect cow pupil size in **Paper I**. A melanopsin-driven function could explain this, since pupillary constriction to steady light is primarily mediated through ipRGCs. However, these light-sensitive ganglion cells also receive input from retinal cones and rods (Dacey *et al*., 2005; Weng *et al*., 2013). Since the pupil remained dilated even with almost a 100-fold increase in photon flux, a higher number of photons reached the retina. As the pupil remained dilated even with increased light intensity, the cow's eye appeared to perceive red light as dimmer than blue or white light. This supports previous suggestions that the input from the ML-cones to the ipRGCs has less impact on the pupillary light reflex than shorter wavelengths detected by the S-cones and/or ipRGCs (St Hilaire *et al*., 2022).

6.1.1 Red or blue light

When red light with PFD 34.9 µmol m^{-2} s^{-1} was used in **Paper I**, the diurnal release of melatonin was similar to that in cows exposed to blue or white light. Some farmers use red light as night lighting to observe cows without disturbing them. The light intensity at which the melatonin level starts being affected by red light is still unknown. In **Paper I**, the high intensity of red light applied probably made the signal from the ipRGCs as strong as with other lights. This high red light intensity was chosen to provide good working conditions for the light lab staff. When choosing red and blue light for the lab, the blue light appeared brighter to the human eye than the red light. Therefore red light intensity was considered a limiting factor and was set to a level that enabled staff to perform their daytime work duties, while the blue and white light intensities were set to match the red light in terms of PFD.

Blue light has been suggested to have a carry-over increase in activity, and therefore can have negative impacts on human night sleep (Tosini *et al*., 2016). In **Paper I,** there were no differences in milk production, DMI or activity between the blue light and other light treatments. However, activity was only measured as standing or lying in that study, since the light lab was a tie-stall, and it is possible that blue light would increase activities such as moving around in a loose housing system. Increased DMI at night when a lower light is applied and a less pronounced diurnal rhythm in the herd as a whole would be beneficial on AMS farms (Deming *et al*., 2013), as it would provide a combination of even cow traffic and at the same time a break from artificial light.

6.1.2 Stress indicators

Darkness has been suggested to increase stress or make cows more hesitant (Phillips & Morris, 2001; Stookey *et al*., 2007), where hesitation can be interpreted as cows being more fearful or cautious of their surroundings. There are concerns that this may encourage farmers to supply a high light intensity also at night, thereby increasing energy costs, preventing the cows from having a break from artificial light and potentially also eliminating the stimulation of long daylight photoperiod on milk yield. Therefore, **Paper II** investigated the effects of darkness and dim light intensities on cow heart rate, respiratory rate, vocalization and frequency of defecation, as all of these are commonly used as stress indicators (Grandin, 2001). The cows in that study did not show any signs of stress, either in darkness or any of the dim lights tested, and they had no problems passing through the obstacle course without bumping into things. This suggests that supplying night light derives more from what people need when moving around in a dairy barn than what the cows need or that we are projecting human emotions and behaviors onto the cows.

6.1.3 Animal welfare

Animal welfare is an important aspect in the use of light and darkness for cows, but it was not studied specifically in this thesis. Interestingly, assumptions on the effect of light and dark on cow welfare seem to be based on research in other species, such as humans or mice. Several physiological effects of light on dairy cows were detected in this thesis, and it would be possible to draw conclusions on welfare aspects based on those data. However, more research with the cow as a model is needed, since otherwise, the conclusions would be based on a small sample with other species as reference. Consideration of the light environment and how it is measured and described is essential in all animal research.

6.2 Daytime lighting

Cows maintained daily milk yield in red, blue and white light in **Paper I,** although milk yield can be expected to decline slowly after peak lactation Knight, 2001). In the herd studied in **Paper I** the expected decrease was 2 kg milk per week, but this decrease did not occur. There are several possible explanations for the maintained lactation levels in that study. Firstly, higher amount of photon flux from the LED lights, regardless of color, may have stimulated higher milk yield. Secondly, a LDPP was applied and earlier studies have shown that this increases milk yield compared with a natural day photoperiod (NDPP) (Dahl *et al*., 1997). Thirdly, the cows in the light lab were tied in stalls with *ad lib* access to feed without competition, whereas they are normally housed in a loose housing with competition over eating spaces. However, the maintained DMI and milk yield is an intriguing finding and the effect of higher photon flux in daylight should be evaluated further in future studies.

6.2.1 Day light color

Artificial light in dairy barns usually includes blue light, since white light is a mixture of blue, green and red wavelengths. White light is the most comfortable light for humans to work in (Cajochen, 2007) and the most common choice of light for dairy barns, but the different mixtures of wavelengths in white light could result in some color variations. The types of white light used in **Papers I** and **II** were perceived as similar by a human trichromat, but there were differences in their spectral distribution. This shows the importance of reporting both the spectral distribution and the intensity of the light used in animal housing studies. While the effects of any differences in the white light may be too small to detect, a study comparing the effects on metabolism and physiology in mice of blue-enriched LED and fluorescent lighting found differences in several factors between these two types of white light (Dauchy *et al*., 2019).

Light environment during daytime differs between dairy farms and mean light intensity is often lower than the recommended value, as shown in **Paper III** and as reported previously by Reksen *et al*. (1999). This may have a particularly strong effect in the Nordic countries, due to the short natural day length during winter. Improving light management in dairy barns is fairly easy to achieve and the greater use of electricity during the day could to some extent be compensated for by applying a lower level of light at night than is commonly used today.

6.3 Night-time lighting

Suitable night-time lighting depends on the target user. For animal caretakers, there are often regulations regarding a safe work environment, but it should be possible for staff to check the cows without switching on full daylight lighting. An abrupt change from dim light to bright light may create a stressful situation for the cows. It is also possible that interruptions in the low night light period by turning on the day light can disrupt the diurnal rhythm of the cows. The level of melatonin in the blood of dairy cows changes rapidly when the light intensity increases, as the results in **Paper I** show. On most nights, staff would probably not need to enter the barn. However, some sensors and other equipment in modern barns may rely on light to function, and it is important that such technology is designed to work with infrared light, rather than light that affects the animals. Not supplying light during the night also saves energy.

6.3.1 Darkness during the night

According to the results in **Paper II**, cows do not need artificial light during night-time, as they are able to navigate accurately in in a room without inlet of natural light and no artificial light. The lack of difference between the darkest light environment and any of the other light treatments in **Paper II** is interesting, since Phillips *et al*. (2000) found that cows walked faster in darkness than in brighter light. Discrepancies in study design between **Paper II** and Phillips *et al*. (2000) may explain the observed contrasts in walking speed, *e.g*. Phillips *et al*. (2000) used a passageway while in **Paper II** an arena that was not part of the cows' regular housing unit was used. Moreover, the obstacles in the test arena probably prevented the cows from walking at high speed, in both darkness and at bright light intensities; the cows walked slower in all light treatments than what they did in the study by Phillips *et al*. (2000).

A low level of light at night may be important to achieve appropriate resting periods, which are generally seen at night rather than during the day (Kilgour, 2012). In practice, night lighting differs between farms, both in terms of length of the darker period and light intensity (Reksen *et al*., 1999). The night light measured on Farm C in **Paper III** was of low intensity and low uniformity. However, measurements were only made in the lying area on that farm, since it was separate from the feeding and milking area. The lighting schedule on Farm C involved having all light fixtures on during the day and only three on during the night, with the latter making some lying stalls very bright and some areas very dark. It may be better to dim all light fixtures, creating a uniform low-light environment at night.

In **Paper I**, low-intensity dim white light was used at night regardless of daytime light treatment, because the focus was on daytime lighting and eventual carry-over effects of blue light to night time. Since the difference between daytime and night-time light intensity was high, use of low-intensity dim white light, instead of no lights, probably did not affect the results of diurnal rhythms.

6.3.2 Night light color

Paper II investigated the effects of cow movements in red or white light and found no differences between the spectral distributions when the light had high uniformity and high intensity (above 0.65 μmol m^{-2} s^{-1}). In red light with low intensity, the cows walked faster with fewer strides, which is reported to occur when cows are comfortable walking on a surface (Phillips & Morris, 2001). The floor surface did not differ between the treatments in **Paper II**, implying that the effect of a faster walking pace was attributed of the light environment and the obstacle course.

Red light with low uniformity and with intensity ranging from 0.01 to 0.46 μmol m^{-2} s^{-1} made the cows in **Paper II** walk more slowly and spend more time discovering their surroundings. This light level may be on the verge of bovine mesopic vision, where rod photoreceptors are close to saturation and cones are still weakly stimulated (Ofri & Ekesten, 2021), meaning that it can be difficult for cows to distinguish objects or interiors.

6.3.3 Characterization of darkness

It is difficult to characterize the light intensity during dark periods. Most previous studies mention allowing a dark period within each 24-h cycle (Suarez-Trujillo *et al*., 2020; McCabe *et al*., 2021) and some also report measured light intensity during darkness of 0 lx (Phillips *et al*., 2000). However, it is challenging to assess the actual level of darkness, since a lux meter does not have the necessary sensitivity at low photon flux, and some photons may have been available within the room at 0 lx. In **Paper II**, there were difficulties measuring the level of darkness in dark periods, as one of the instruments (PAR200) showed better absolute calibration than the other (JAZ spectrophotometer), but was still not sensitive enough to measure the lowest light levels. In addition, measuring outside the light lab environment, for example in dark farm barns, presents further challenges. For example, using the JAZ spectrophotometer in a barn with cows, as done in **Paper III**, is complicated since the device needs be connected by cable to the mains and to a computer while measuring. The dust and moisture normally present in cow barns pose again more challenges to the measuring equipment.

6.4 Lighting design

Light environments for dairy cows differ depending on the production system and building design. Ideally, the indoor light environment should perhaps resemble the outdoor light environment, with a bright sky, a dark shift at the horizon and dark ground. One way to create a brighter indoor 'sky' is to direct the light fixtures towards a light-colored ceiling (Makaremi *et al.*, 2019). Indirect lighting creates a uniform indoor light environment, although it increases the energy consumption for lighting (Makaremi *et al*., 2019). A light-colored ceiling in dairy barns would also require regular washing to maintain high light reflectance.

There are many factors to consider when designing light intensity in animal barns. Too high light intensity (*e.g.* glares) could cause discomfort, to people (Hopkinson, 1972) and cows and affect cow movements (Willson *et al*., 2021). However, the interior reflectance is usually low in barns, due to dull colors of interior surfaces, which makes bright light intensities appear less bright than in a white room. With available technology, it is possible to adjust the artificial light indoors to the outdoor light, *e.g*. the light fixtures could be dimmed during a bright sunny day without compromising the indoor light environment. Dimming the light fixtures during bright days would also save energy.

How light is characterized, *e.g.* how the sensor is positioned when measuring the light, can also affect the interpretation when choosing the design of lighting systems. The ELF method describes the light environment based on the position of the animal's eyes, and the images face horizontally. Measurements with an Lx meter and/or spectrophotometer were made with the sensor directed towards the ceiling, before deciding on the light levels tested in this thesis. This approach enabled us to reproduce the measurements easily and to create a uniform light environment. We thought of it as measuring the light intensity at a cow's forehead instead of trying to measure the possible light reaching the eye.

6.4.1 Interior colors

The interior colors in a barn can affect the light environment, with color and reflectance off surfaces probably affecting cow vision. Cow barn interiors at the farms included in **Paper III** were dark in color. An additional consideration is floor color, since it has been found *e.g.* that a reflecting floor makes sheep more hesitant to move (Hutson, 1981). The floors in barns are usually not white, but it may be good to keep in mind when designing light environments that floors with high reflectance of light should be avoided.

6.4.2 Uniformity

The uniformity of light is rarely mentioned in studies examining the impact of light on dairy cows. However, software for designing indoor light environments often simulate the environment and light uniformity using a blueprint of the building and data on the height of light fixtures, as done in the test arena in **Paper II**. A weakness in most available software is the lack of consideration of impacts of interiors on the light environment at cow eye level. For example, in modern dairy barns rails can be used for feed or bedding material distribution. These rails are commonly placed above the cows and below the light fixtures, creating shadows that are not accounted for by the software. In addition, daylight inlets together with clear, sunny weather can create sharp contrast shadows within a barn and possibly hinder cow traffic (Willson *et al.*, 2021).

During the night, it is common practice on dairy farms to leave only a few light fixtures on and turn off the rest, as seen on Farm C in **Paper III**. This results in a light environment with low uniformity and most likely creates shadows within the barn. The effect of uniformity was tested in **Paper II** and the results showed that the cows were not affected by low uniformity of white light. However, the study was performed in an arena with obstacles, where deviations in behavior could be difficult to detect, although some trends were observed.

6.5 Challenges with light studies

Creating a light lab eliminates most external factors that could affect the results of the study. Though, cows are animals that like a fixed routine (Jacobs & Siegford, 2012) and a move to another barn or room could influence their behavior and performance. An acclimatization period was therefore applied in all trials in the light lab.

It would be ideally for cow comfort and performance to remain similar, the light lab should therefore have been established in the regular barn environment. However, the main barn has daylight inlets, so the actual effect of the light treatments would have been more difficult to investigate. Other studies have covered windows with light-proof curtains and have blocked openings around doors (McCabe *et al*., 2021).

Creating the optimal study is challenging and there is always room for improvement. Using a light lab made it possible to evaluate the effect of the artificial light regimes without disturbance from any outdoor light, although it would be interesting to see the effects of the lighting systems in the cow's everyday environment.

7. Conclusions

The overall conclusions from the work in this thesis are that:

- Characterization of the light environment in dairy barns is challenging. Structured methodical measurements are preferable, since a single or a few measurements will give an incomplete picture of the actual light environment. A spectrophotometer that measures photon flux density within the visible light spectrum should be the first choice, instead of a lux meter adapted to human photopic vision.
- Spectral composition of light (red, blue and white light with intensity of 34.7 to 36.9 µmol m^{-2} s^{-1}) did not affect milk production or lying time.
- A dark environment, without any supplementary light, did not change the walking behavior of cows passing through an obstacle course.
- Cows reduced walking speed when subjected to non-uniform, low-intensity red light, indicating the importance of avoiding non-uniform light in dairy barns.
- Pupil constriction in the cow's eye limited the effects of light intensity above 23 µmol m^{-2} s^{-1} when wavelengths between 400 and 600 nm dominated the spectral composition. When subjected to red light, at 600-700 nm, the cow's pupil did not constrict with increased light intensity.

8. Practical implications and future prospects

Changing old light fixtures to LEDs lowers energy consumption and LED fixtures also last longer. Such a change could also improve the light environment by providing a light spectrum suitable for a specific indoor environment, considering colors, location and interiors when designing the system. However, there are still many concerns to investigate regarding light design and placement of light fixtures.

Light at night may be comforting for barn staff, but not necessarily for the cows. Turning the lights off would instead give the animals a break from artificial light and could promote the circadian rhythm. The effects of light should also be considered when studying other physiological or behavioral factors, since a disrupted circadian rhythm may affect the results.

It would be interesting to test the light intensity of red light and its impact on melatonin secretion in dairy cows, starting with low light intensities and step-wise increasing the intensity. It may be possible to provide red light at night without disturbing the cow's circadian rhythm in terms of melatonin production, but the threshold light intensity should be determined. The human eye functions well in red light, but human vision for details is not as great as in white light. Note that when designing red night light for a barn a lux meter cannot be used to decide the intensity, since its range of measurement is based on human photopic vision.

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Popular science summary

During the late 1800s, Swedish agronomist Hjalmar Nathorst recommended windows and lighting in dairy barns, to improve the indoor environment for farmers and also animal health. Later studies found that extending the natural day with artificial light, to a total of 16 or 18 hours of daylight, increases milk production by approximately 0.5 to 3.3 kg daily. Interestingly, a constant light regime does not increase milk yield, so a dark period is required for this benefit to be realized. Animal legislation in some countries requires cows to be allowed a break from artificial light at night. In contrast, the Swedish animal welfare act states that night light is mandatory for cattle, but does not specify the light intensity to be used at night. In cows allowed to choose freely how to allocate their time, behavior differs between day and night. For example, cows often spend more time resting and lying down at night and more time performing active behaviors during the day. In systems where cows are milked by robot, it is important to have a steady flow of cows to the robot at all hours. Therefore some farmers keep the light on day and night in order to maintain a high activity level among the cows throughout the night and avoid fewer visits to the milking robot when it is dark in the barn.

Once light-emitting diode (LED) light fixtures became available, they quickly attracted the interest of farmers, as LED fixtures last longer and use less energy. Using LED, it is also possible to create light of different colors, a feature that has been exploited by manufacturers promoting *e.g.* blue light to increase activity and red light at night to enable staff to monitor animals without disturbing them. However, there is currently little scientific evidence to support these claims. Therefore, this thesis investigated the impact of different light environments on dairy cows by varying the intensity, color and distribution of light provided in the barn during the day and at night.

Three studies were conducted in a light-proof barn at SLU's research facility at Lövsta, to assess the effect of different light treatments without the additional influence of outdoor light. The first study investigated whether cow pupil size changed when the intensity of the light provided (red, blue or white light) was increased stepwise from dim to bright (resembling conditions on a slightly cloudy day outdoors). In white and blue light, pupil size decreased when the light intensity was increased. However, pupil size remained large in red light, even when light intensity increased. This indicates that uniform red light (in contrast to blue and white light) provides poor stimulus for the retinal cells driving pupillary constriction in cows.

The second study investigated the effect of different colors of indoor daytime light on milk production, cow activity, feed intake and daily rhythm. The colored light (blue, red and white) was provided between 5 am and 9 pm, while during the night dim white light was supplied. Milk production did not differ between the different colored light treatments and the level was maintained throughout the five-week study period, regardless of daytime light color. This suggests that the light intensity and light program used were more important than the color of the LED light. The impact of light color on the daily rhythm in cows was assessed by measuring levels of the hormone melatonin in blood samples collected on four occasions during the day (8.30 am, 4 pm, 11.30 pm and 4 am. Melatonin, also known as the sleep hormone, is involved in regulating the biological clock in mammals. The color of light did not affect the levels of melatonin in blood but, as expected, levels were higher in samples collected at night than in samples collected during the day.

The third study tested the ability of cows to navigate an obstacle course in different light environments. After being trained to walk through the obstacle course, cows were tested in darkness and at increasing intensity of either white or red light, in a total of 14 different light treatments. No difference in ability to navigate was seen when cows walked through the obstacle course in darkness compared with bright white light. However, under dim red light where parts of the obstacle course were illuminated better than other parts, the cows walked more slowly and seemed to investigate their surroundings more closely. This suggests that cows may have less problems navigating in darkness than in a relatively dim environment where some areas have more light than others.

In an additional study conducted on four Swedish dairy farms, the light environment in barns was assessed by measuring different properties of light.

Using only one standardized method for these measurements proved to be difficult, since the building outline, interior and prerequisites differed significantly between the farms, so several methods were applied to capture the light environment. The overall results showed that the indoor light environment differed significantly between and within the farms. Most interestingly, the light distribution, known as light uniformity, in the measured areas on all farms was lower than the level recommended by EU regulations for a workplace. Based on the previous findings for cows navigating an obstacle course, this low uniformity may impair the ability of cows to move freely around in the barn. Using methods other than a lux meter to measure light provided a more nuanced description of the light environment, information that is needed when designing light environments adapted to cows.

In summary, cows do not necessarily need light to find their way around in the barn at night and light color may be secondary in importance to light intensity and uniformity. When designing the light environment in dairy barns, it is important to include daylight inlets, interior surfaces and their color, and the building outline.

Populärvetenskaplig sammanfattning

I slutet av 1800-talet rekommenderade agronom Hjalmar Nathorst fönster och tillgång på dagsljus i mjölkladugårdar. En ljus ladugård sågs som en förbättring av inomhusmiljön, personalens arbetsförhållanden och djurhälsan. Nästan hundra år senare visade forskare att mjölkproduktionen kunde öka med 0,5 till 3,3 kg dagligen om den naturliga dagen utökades med hjälp av artificiellt ljus till totalt 16 eller 18 timmars dagsljus. Men om det är konstant ljus under 24 timmar kommer mjölkproduktionen inte att öka, eftersom det behövs en mörk period varje dygn för att effekten ska nås. I vissa länder är det lagkrav att kor ska få vila från belysning under natten, men enligt den svenska djurskyddslagen är nattbelysning obligatoriskt för nötkreatur. Lagtexten specificerar dock inte vilken ljusintensitet som ska användas på natten och inte heller på dagen. I produktionssystem där korna mjölkas av en mjölkningsrobot är det viktigt att aktiviteten i lagården är jämnt fördelad över dygnet så att flödet av kor till roboten är detsamma över hela dygnet. Kor vilar gärna på natten och är aktiva på dagen, men i praxis önskar många lantbrukare att kunna motverka detta genom att ha ljuset i lagården tänt hela dygnet.

Sedan LED-armaturer blev tillgängliga har de fångat lantbrukares intresse eftersom LED håller längre och använder mindre energi. Med hjälp av LED är det också möjligt att skapa ljus i olika färger, något som utnyttjats av tillverkarna. Försäljningsargument för LED har bland annat varit att blått ljus ökar aktiviteten hos korna, och att rött ljus kan användas under natten så att personalen kan övervaka djuren utan att störa dem, eftersom det röda ljuset inte uppfattas som ljus av kor. Dock saknas tydliga vetenskapliga bevis för att dessa påståenden stämmer. Syftet med den här avhandlingen var att undersöka hur ljusmiljön påverkar mjölkkor genom att testa olika ljusintensiteter, färger och jämn eller ojämn fördelning av ljuset, men också att använda flera olika metoder för att mäta och bättre beskriva ljusmiljön.

Tre studier har genomförts vid SLU:s forskningsanläggning Lövsta Lantbruk. Dessa tre studier utfördes i en ladugård där ljuset kunde kontrolleras i detalj, för att säkerställa att effekten av LED-belysning testades utan att rummet påverkades av ljus utifrån. Den fjärde studien genomfördes på fyra gårdar med mjölkproduktion.

I den första studien visades att pupillen inte reagerar på samma sätt på ökande ljusintensitet när ljuset har olika färg. I vitt och blått ljus minskade pupillstorleken när ljusintensiteten ökades, medan pupillerna förblev stora i rött ljus, även när ljusintensiteten ökade. Det här kan betyda att rött ljus inte stimulerar de näthinneceller som skickar signaler så att pupillen drar ihop sig, i lika hög grad som vitt och blått ljus.

I den andra studien testades rött, vitt och blått dagsljus (kl. 05-21) med samma mängd ljus för alla färger, och på natten (kl. 21-05) användes ett svagt vitt ljus. Studien pågick under fem veckor, och varken mjölkmängd eller dygnsrytm påverkades av färgen på dagsljuset. Däremot var mängden mjölk som varje ko producerade konstant under hela studieperioden trots att studien utfördes i en fas då kornas mjölkmängd förväntas minska. Det här är intressant eftersom det tyder på att ljusintensiteten och ljusprogrammet är viktigare än den faktiska färgen på LED-ljuset. Effekten av ljusets färg på den dygnsrytmen utvärderades genom att mäta nivåer av hormonet melatonin i blodprover som tagits vid fyra tillfällen under dygnet; kl. 08.30, 16.00, 22.30 och 04.00. Melatonin är ett sömnhormon och är involverat i att reglera vår biologiska klocka. Ljusets färg påverkade inte nivåerna av melatonin, men som förväntat var nivåerna högre i prover som samlades in på natten jämfört med de prover som samlades in under dagen.

I den tredje studien gick korna igenom en hinderbana i olika ljusmiljöer, både ljusa och mörka miljöer, men också i jämnt och ojämnt ljus, och i ljus med olika färg. Korna gick lika obehindrat igenom banan i mörker som i starkt vitt ljus. I svagt rött ljus där vissa delar av hinderbanan var bättre upplysta än andra, gick korna dock långsammare och verkade undersöka sin omgivning närmare. Slutsatsen från den här studien är att kor ser bra i mörker men är mer osäkra i en ganska mörk miljö med ojämn belysning.

Den fjärde studien genomfördes på fyra svenska mjölkgårdar. Syftet var att beskriva ljusmiljöer på gårdar genom att mäta ljus med olika metoder. Att använda endast en mätmetod visade sig vara svårt eftersom gårdarnas byggnader, inredning och förutsättningar skiljde sig markant, istället användes flera olika metoder. De övergripande resultaten från de fyra gårdarna bekräftar vad man sett i tidigare, liknande undersökningar: att ljusmiljön skilde sig markant mellan och inom gårdarna. Mest intressant var att på alla gårdar uppmättes väldigt ojämnt ljus, och på ingen av dem nådde ljusmiljön upp till vad som rekommenderas för en arbetsplats för människor. Att använda andra mätinstrument än enbart en luxmätare gav en förfinad beskrivning av ljusmiljön, vilket underlättar för att skapa ljusmiljöer anpassade till kor.

Sammanfattningsvis behöver inte kor speciellt mycket ljus för att hitta i ladugården under natten, färgen på ljuset verkar dessutom vara mindre viktig än ljusets intensitet och uniformitet (jämna fördelning över ytan). När ljusmiljön i en ladugård utformas bör dagsljusinsläpp, ljusarmaturer och deras placering, inredningen och färger, samt byggnadens placering med hänsyn till väderstreck och omgivningen, tas med i beräkningen.

As sure as night is dark and day is light
I keep you on my mind both day and night
And happiness I've known proves that it's right
Because you're mine, I walk the line
- Johnny Cash

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I

RESEARCH ARTICLE

Effects of achromatic and chromatic lights on pupillary response, endocrinology, activity, and milk production in dairy cows

Sofia Lindkvist[1]*, **Emma Ternman**[1], **Sabine Ferneborg**[2], **Daniel Bånkestad**[3], **Johan Lindqvist**[3], **Björn Ekesten**[4], **Sigrid Agenäs**[1]

1 Department of Animal Nutrition and Management, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden, **2** Department of Animal and Aquacultural Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway, **3** Department of Horticulture and Technology, Heliospectra AB, Gothenburg, Sweden, **4** Department of Clinical Sciences, Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, Uppsala, Sweden

* Sofia.lindkvist@slu.se

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Abstract

Artificial light can be used as a management tool to increase milk yield in dairy production. However, little is known about how cows respond to the spectral composition of light. The aim of this study was to investigate how dairy cows respond to artificial achromatic and chromatic lights. A tie-stall barn equipped with light-emitting diode (LED) light fixtures was used to create the controlled experimental light environments. Two experiments were conducted, both using dairy cows of Swedish Red and light mixtures with red, blue or white light. In experiment I, the response to light of increasing intensity on pupil size was evaluated in five pregnant non-lactating cows. In experiment II 16h of achromatic and chromatic daylight in combination with dim, achromatic night light, was tested on pregnant lactating cows during five weeks to observe long term effects on milk production, activity and circadian rhythms. Particular focus was given to possible carry over effects of blue light during the day on activity at night since this has been demonstrated in humans. Increasing intensity of white and blue light affected pupil size ($P<0.001$), but there was no effect on pupil size with increased intensity of red light. Milk yield was maintained throughout experiment II, and plasma melatonin was higher during dim night light than in daylight for all treatments ($P<0.001$). In conclusion, our results show that LED fixtures emitting red light driving the ipRGCs indirectly via ML-cones, blue light stimulating both S-cones and ipRGCs directly and a mixture of wavelengths (white light) exert similar effects on milk yield and activity in tied-up dairy cows. This suggests that the spectral composition of LED lighting in a barn is secondary to duration and intensity.

Introduction

In dairy cows, photoperiod can be used as a management tool to increase milk yield and improve working conditions for barn staff. When artificial light is used to extend a natural 8-h

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day to 16 h of daylight for lactating cows, milk yield [1, 2] and circulating insulin-like growth factor-1 (IGF-1) increase [3]. It is not known whether the type of light is important for the galactopoietic response, but manufacturers of light-emitting diode (LED) fixtures for dairy barns suggest that specific wavelengths are important for the effect on milk yield. Red light is often promoted by the industry as night light, because it is claimed not to affect the cows' diurnal rhythm.

LEDs reduce consumption of electricity for illumination in dairy barns and require less maintenance compared with several other types of light fixtures available for animal houses, which makes them increasingly popular. The use of LEDs also entails better control of light intensity as the diodes can be dimmed, as well as better control of the light spectrum as there are many different color types available. Artificial light supplements daylight, when daylight is available, and provides adequate levels of illuminance during the rest of the day to allow a daylight-like environment of 16h per 24h for lactating cows [4]. Humans respond differently to natural light compared to artificial light [5], and little is known about how cows respond to lights of different spectral composition. With the increasing use of LED light on dairy farms, it is interesting to investigate whether specific wavelength mixes are beneficial for increased milk production.

Mammals, including cattle have two major types of photoreceptors, cones and rods, that are involved in vision [6]. Cattle, like most mammals, are dichromats and have short-wavelength-sensitive (S-cones) and medium- to long-wavelength-sensitive cones (ML-cones) with opsins peaking at 451 (blue) and 555 (greenish-yellow) nm, respectively [7]. However, the eye also provides sensory input for non-image-forming visual functions, including circadian photo entrainment for setting internal biological clocks, inhibition of melatonin release, which plays a pivotal role in the sleep-wake cycle, and adjustment of the number of photons reaching the retina through the pupillary light reflex [8–11]. A third group of photosensitive receptors in the retina, intrinsically photosensitive retinal ganglion cells (ipRGCs) containing the photopigment melanopsin, drive or contribute to regulation of all these functions [9, 12–15].

In humans, low light exposure during night-time causes acute suppression of melatonin [16]. Studies in humans have shown that the most potent part of the spectrum for providing circadian input for regulation of melatonin secretion is around 446–477 nm [13, 17, 18]. These wavelengths coincide with the absorption peak of the bovine S-cones (451 nm) and are also close to the peak absorption maximum of melanopsin [9, 12, 13]. There is also substantial evidence that exposure to blue light can increase alertness and stimulate cognitive function in humans [19], also after the blue light is turned off [20, 21]. In dairy calves, blue LED light suppressed the expected melatonin increase in the evening when compared to another treatment with yellow LED light [22]. It is therefore possible that using blue LED light during daytime, or during part of the day, could increase the activity of cows at night also when the lights are considerably dimmed or turned off to allow the animals a break from artificial light.

Furthermore, the incident of photon flux onto the retina is adjusted by the pupil size. For a long time, constriction of the pupil in daylight was considered to be driven by retinal cones and chiefly related to the luminance. More recently, it has been postulated that although the photoreceptors play a role in regulation of pupil size at least when there is a transient change in background light, the size of the steady-state pupil is mainly controlled by the ipRGCs [23, 24]. Pupillary dilation is almost completed at one minute in humans and fully completed in 10 minutes at low light levels [25], whereas pupillary constriction is a very rapid process and retinal cone adaptation also seems to be completed in less than 10 minutes [26]. Hence we decided to study the pupil size in cows under different lighting conditions to understand if pupil size and thereby retinal illumination changed when different lighting regimes were used.

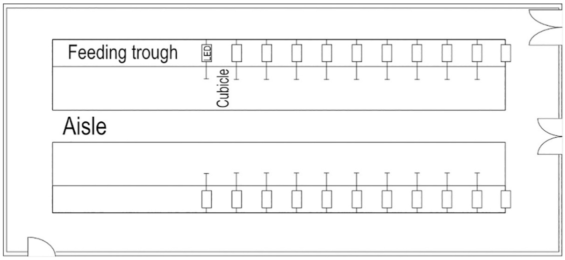

Fig 1. Layout of the Light lab. LED fixtures placed on each side of the head of every cow.

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The aim of this study was to investigate the effects of different spectral compositions of artificial light on lactating dairy cows. Specific hypotheses were that: i) Pupil response is driven by photon flux and does not differ between different wavelengths; ii) blue light during the day increases the activity of cows at night; and iii) red light does not support diurnal release pattern of melatonin as well as blue or white light.

Material and methods

The study was conducted at the Swedish Livestock Research Centre, Uppsala, Sweden, and comprised two experiments. All animal handling was approved by the Uppsala Ethics Committee for Animal Research, Uppsala, Sweden (reference no. 5.2.18-11064/16).

The experiments were performed in a tie-stall barn with a controlled light environment and no contamination from external light (hereafter called the 'Light lab'). The Light lab had tie-stalls in two rows, on each side of an alley. One light treatment could be applied per row, allowing two treatments to be tested at a time. The tie stalls had rubber mats and wood shavings as bedding material. The stalls were cleaned and bedding material replaced during milking. Water was provided *ad libitum*, from individual automatic water bowls. The Light lab was equipped with LED light fixtures (Elixia LX602G, Heliospectra AB, Sweden) placed on each side of the head of every cow, approximately 140 cm above the forehead (Fig 1). The LEDs in the light fixtures were remotely controlled and hence, both intensity and the spectral composition of the light could be adjusted.

Light measurements

Light was measured at the level of the cow eye, approximately 125 cm above the floor, with a photosensor directed towards the ceiling. A luxmeter [Hagner Screenmaster, B. Hagner AB, Solna, Sweden], a photometer [(IL-1700, International Lights, Peabody, MA, USA], and a spectrometer [Jaz, Ocean Insight, Inc. Dunedin, Florida, USA] were used for this purpose, and hence illuminance (lux), luminance (cd/m^2), photon flux density (μmol s^{-1} m^{-2}), and light spectrum (μmol s^{-1} m^{-2} nm^{-1}) were quantified. To simplify reporting, we frequently use the expression 'light intensity' rather than these four physically correct terms when referring to amount of light in the barn, and we refer to the different mixtures of wavelengths used in the experiments as 'colors' based on the hues a normal human trichromat would perceive on seeing the light (Table 1 and Fig 2). The different intensity levels tested (1–10, Table 1) were designed to provide similar photon flux density, while the illuminance and luminance values were used for comparison.

Table 1. Light intensity levels used in the experiments, expressed as photon flux density (μmol s^{-1} m^{-2}), illuminance (lux), and luminance (candela/m^2).

Light	Red			Blue			White		
Intensity	Photon flux density	Illuminance	Luminance	Photon flux density	Illuminance	Luminance	Photon flux density	Illuminance	Luminance
1	-	-	-	0.18	1.7	2.2	0.18	11.7	1.87
2	0.36	6.1	0.005	0.4	1.8	4.8	0.4	32	4.8
3	0.83	14.8	0.06	0.73	2.4	12.1	0.62	49.1	9.3
4	1.46	21.4	0.12	1.4	47	24	1.48	115.6	23.7
5	2.78	40.6	0.19	2.85	94	42.1	2.83	219	42.9
6	5.89	86.6	0.49	5.82	127	75.5	5.82	390	85.9
7	11.3	167.4	0.83	11.4	370	158.5	11.6	662	135.7
8	23.4	342	1.72	23.0	773	324	23.1	1070	216
9	34.9	676	31.8	34.7	1674	416	36.9	1668	325
10	-	-	-	-	-	-	46	3550	745

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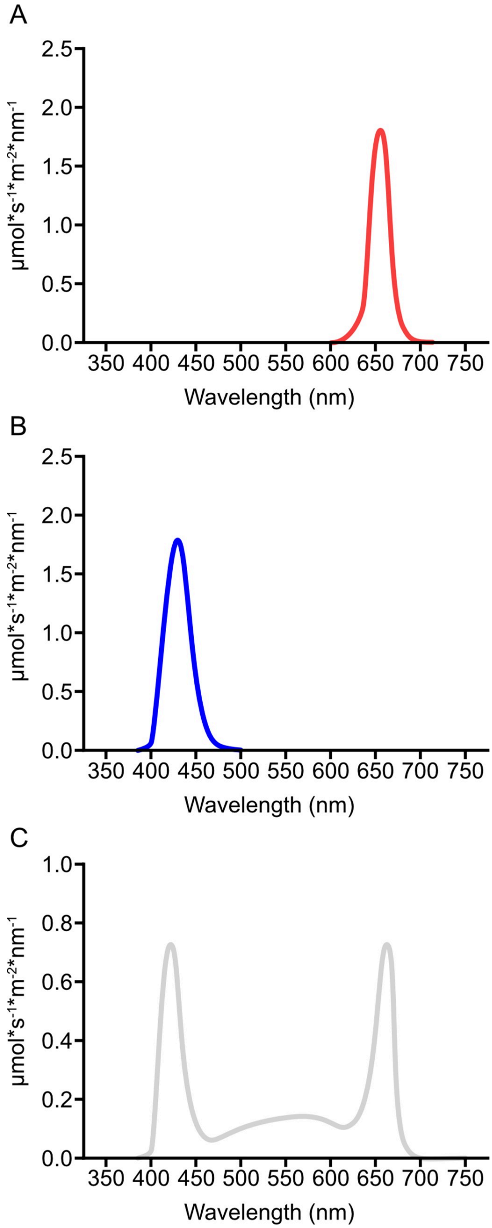
Experiment I

Size of pupils in response to blue, red, and white light of increasing intensity was studied in five pregnant non-lactating cows of the Swedish Red (SRB). The exposure started with the dimmest light ($Blue_1$, followed by $White_1$, and then Red_2, $Blue_2$, $White_2$) and the light intensity was increased step-wise as shown in Table 1. When the cows had adapted to the test light for 10 minutes, photographs of each eye were taken at each light intensity at approximately 2–3 m distance, using a digital camera (Nikon D800 with a Nikon AF-S Nikkor 70-200mm f/2,8 lens). Relative area of the pupil (RAP) was calculated as the area covered by the pupil in the photograph divided by the area circumscribed by the peripheral iris at the *limbus cornea* (Fig 3). To ensure a comparable scale in the photographs, a piece of white surgical tape with a centimeter scale was placed below the eye on every cow. All photographs were analyzed by the same researcher (author S.L.) using imaging software (Adobe Photoshop 2020 version 21.0.3). Cow identity and lighting conditions for each image were blinded for measurements. To estimate the amount of light actually reaching the retina and to enhance comparison with conventional retinal illumination measured in Trolands (which is equal to the pupil area in mm^2 times the luminance in candela/m^2) [27], the photon flux was multiplied by mean RAP.

Experiment II

Forty lactating SRB cows were blocked according to days in milk (range: 117–331), days in pregnancy (range: 31–137), parity (range: 2–7), and daily milk yield (range: 22–45 kg) and randomly assigned to one of two light treatments in each of two periods: Blue (n = 10) and Red (n = 10) in period 1, and White (n = 10) and White-Blue (n = 10) in period 2. Period 1 ran from January to March 2019, and period 2 from March to May 2019.

A long-day photoperiod (LDPP) was used, with 16 h daylight and 8 h of dim night light (Fig 4). The cows were moved into the Light lab 22 days prior to the onset of the light experiment, to allow them to acclimatize to the surroundings. Thereafter the treatment period started and only LED lighting was employed for 33 days. During period 1, the daylight intensities were $Blue_9$ and Red_9, respectively. During period 2, $White_9$ was used as the White light treatment, while for the White-Blue treatment, $White_9$ was turned on for 10 h and switched to $Blue_9$ for the last six hours of the daytime period. All daylight treatments had the same dim night light, $White_1$ (Table 1). The light intensities selected during daytime was a result from experiment I combined with practicalities as ensuring a safe work environment for barn staff.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Wavelength (nm)

Fig 2. Spectral composition of the light used during daytime in the Light lab. (A) Red light treatments, (B) Blue light treatments, and (C) White light treatments.

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Standing and lying behavior. Standing and lying activity was recorded by HOBO Pendant G Data loggers, model UA-004-64 G (ONSET, Bourne, Massachusetts, USA), attached to one hind leg of each cow, throughout the experimental period. Loggers and data were handled using the protocol suggested by UBC AWP [28]. The logger was set to record position (standing or lying) every five minutes. Number of standing and lying observations were summarized first per day (24h), daytime (16h), and night-time (8h), and proportion of standing/lying time was calculated as number of standing/lying observations divided by total observations per day, daytime, or night-time. Number of standing and lying bouts, and bout durations, were also measured, according to standard operating procedures [28].

Feed intake. Silage provided *ad libitum* was replaced daily (0545h) and topped up twice daily (1300 and 1930h). Concentrate was fed four times per day (0545, 1300, 1630, and 1930h), on top of the silage. Every cow had their own feeding through providing individual feed intake. Daily concentrate ration was adjusted to the calculated requirements for individual milk yield according to the NorFor system [29]. Chemical composition of silage, based on samples from the silo and analyzed with near-infrared reflectance spectroscopy, and of concentrate, is shown in Table 2. Silage 1 was fed in period 1 and silage 2 in period 2. In both periods, cows were fed a mix of concentrate 1 and 2. The ratio of the two concentrates were adjusted to ensure an equal crude protein intake. Silage refusals were collected manually before morning and evening feeding for five consecutive days at the end of the treatment period, to ensure feed intake for daytime and night-time, respectively. There was concentrate in the silage refusals on very few occasions. The refusals were weighed, and silage ration was adjusted individually to ensure *ad libitum* feeding. Silage samples were taken from each feeding and stored in a plastic bag at -20° C until analyzed. Samples from two weeks were pooled and analyzed for dry matter (DM)

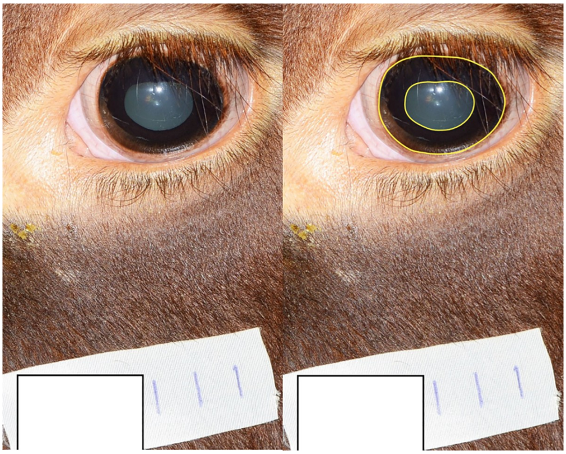

Fig 3. Photograph illustration for the calculation of relative area of the pupil (RAP). The yellow marks in the right photograph indicate the pupil area and the iris area. RAP was calculated by dividing the area covered by the pupil with the area area circumscribed by the peripheral iris at the limbus cornea.

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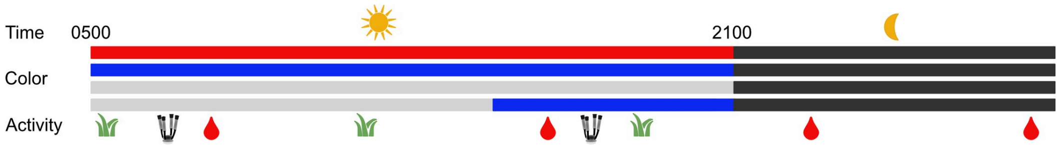

Fig 4. Schematic illustration of the light treatments during 24 hours. Daylight was provided during 16 hours and dim night light during eight hours. Symbols indicate times of feeding, milking, and blood sampling.

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content by first drying at 60˚C overnight, grinding, and then drying at 60˚C overnight [30]. Energy balance was calculated according to the NorFor system [29].

Milk. The cows were milked twice daily at 0615 and 1700h (DeLaval DelPro MU480), and milk yield was recorded automatically. Milk was sampled for five consecutive days at morning and evening milking at the end of the treatment period. Milk samples were obtained throughout milking with the Tru-Test technique (Tru-Test Mechanical Milk Meter (MM6) DeLaval AB, Tumba, Sweden), preserved with 10% bronopol, (2-bromo-2-nitropopane-1·3-diol VWR International AB, Stockholm, Sweden), stored at 8˚C, and analyzed within five days.

Milk samples were individually analyzed for content of fat, protein, lactose, and somatic cell count, using infrared Fourier-transform spectroscopy (CombiScope FTIR 300 HP, Delta Instruments B.V., Drachten, The Netherlands). The mean value for 10 milk samples for each period were used in the statistical analyses. Energy-corrected milk (ECM) yield was calculated based on fat, protein, and lactose content according to Sjaunja *et al.* [31].

Melatonin and IGF-1. Blood was sampled from the tail vein (*v. caudalis mediana*) four times (at 0830, 1600, 2230 and 0400h) during the last 24 hours of the treatment period. The samples were collected in tubes containing Na-EDTA (0.9 x 38 mm; Vacutainer No. 360215; BD; Franklin Lakes, NJ) and placed on ice immediately after collection. Plasma aliquots were obtained after centrifugation for 10 min at 4000 x g and stored at -20˚C until analysis, within one day of sampling. A commercial ELISA kit was used for analyzing melatonin (IBL International 2014) and IGF-1 (Mediagnost 2018). Average sensitivity and intraassay and inter-assay coefficient of variation was 0.09 μg/mL, 2.5%, and 7%, respectively, for IGF-1 (10 assays), and 1.6 pg/ml, 5.3%, and 15%, respectively, for melatonin (13 assays).

Statistical analysis

The mixed procedure in SAS (SAS version 9.4, SAS Institute Inc., Cary, NC.) was used to test whether pupil size was affected by light color (blue, red or white) or light intensity (level 1-9/

Table 2. Chemical composition of silage and concentrate.

Item	Silage 1	Silage 2	Concentrate 1	Concentrate 2
Period 1 (% of diet)	67	-	25	8
Period 2 (% of diet)	-	60	36	5
Dry matter (DM, g/kg)	391	464	880	890
Ash (g/kg DM)	76	114	-	-
Crude protein (g/kg DM)	139	181	170	280
Neutral detergent fiber (g/kg DM)	424	411	260	250
Metabolizable energy (MJ)	10.6	10.7	13.3	14

Both fed in Experiment II to lactating dairy cows exposed to light of different wavelengths during 33 days. Silage 1 was fed in period 1 and silage 2 in period 2 and a mix of concentrate 1 and 2 was fed in both periods to ensure an equal crude protein intake.

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10). Color, intensity, and their two-way interaction were included as fixed effects, and cow nested within treatment as a random effect, with an unstructured covariance structure.

To test whether standing, lying, milk production, feed intake, melatonin, or IGF-1 was affected by light treatment (Blue, Red, White, or White-Blue), the mixed procedure in SAS was used. In all models, treatment and period (first or second) were included as fixed effects, and cow nested within treatment as a random effect, with an unstructured covariance structure. The model for standing and lying also included the fixed effect of time of day (day and night); the model for milk yield, milk composition, and feed intake included the fixed effect of days in milk; and the model for melatonin and IGF-1 included the fixed effect of sampling time (0830, 1600, 2230, 0400h). Interactions of fixed effects were excluded using stepwise backwards elimination; any interaction effect with $P>0.10$ was excluded from the model until all remaining interactions showed $P<0.10$. The two-way interaction of treatment × period was kept in the models for standing, lying, milk yield, milk composition, and feed intake, and the three-way interaction of treatment × period × sampling time was kept in the models for melatonin and IGF-1. Melatonin and IGF-1 were also tested for correlation, both within 24 hours and at the sampling times (0830, 1600, 2230, 0400h), using the correlation procedure in SAS.

Values presented are least squares mean (LSM) ± standard error of the mean (SEM), unless otherwise stated. Results were considered significant at $P \leq 0.05$, while a trend was assumed for probabilities $0.10 > P > 0.05$. Post-hoc means separation for significant main effects was applied using Tukey-Kramer's adjustment of probability values.

Results

Experiment I

There was no significant difference in RAP for the red light intensities tested in experiment I, despite an almost 100-fold increase in photon flux (Fig 5). The average RAP over the entire range of red light intensities tested was 40±1.2%.

In contrast, the brightest blue and white lights produced significant constriction of the pupils, whereas there were no differences for light intensities from 1 to 3 ($p = 1$) in experiment I. On increasing from $Blue_3$ to $Blue_8$, RAP decreased from 39.5±2.1% to 23.5±2.1% ($p < 0.001$) and from 42±2.1% to 27±2.1% using white light ($p < 0.001$). The average RAP for $Blue_9$ was 24 ±2%, for Red_9 38±2%, and for $White_9$ 27.5±2%. In dim night light ($White_1$ used in all the daylight treatments in experiment II), the average RAP was 42±2%. The relative number of photons reaching the retina (RAP x photon flux) for $Blue_9$ was 8.3, for Red_9 13.2, and for $White_9$ 10.0. For the dim night light ($White_1$), the relative number of photons reaching the retina was 0.08 (Fig 6), implying that the relative number of photons reaching the retina during daylight conditions was approximately 100 to 165 times higher than in dim night light.

Experiment II

Standing and lying behavior. Light treatment did not affect cow activity, with an overall standing proportion of 54±1% during daytime and 40±2% during night-time (Fig 7). The overall number of standing bouts was 6±0.4 bouts during daytime and 3±0.2 bouts during night-time. Mean standing bout duration was 78±4 min during daytime and 41±3 min during night-time.

The overall mean number of lying bouts was 8±0.3 bouts during daytime and 4±0.2 bouts during night-time. Mean lying bout duration was 59±2 min during daytime and 55±3 min during night-time.

Feed intake and milk yield. There was no difference in feed DM intake (DMI) between the treatments ($P>0.1$) (Table 3). There was no difference in calculated energy balance

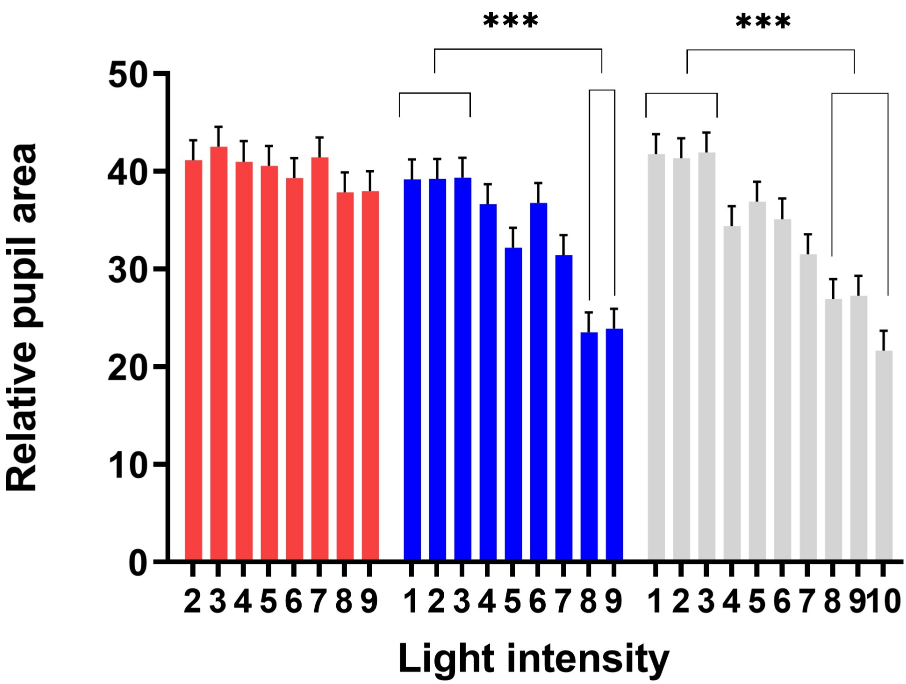

Fig 5. Relative area of the pupil (RAP) of cows exposed to Red, Blue, and White light intensity. There was no difference in relative pupil area for cows under red light. Under blue and white light, the relative pupil area decreased by almost half (***p <0.001).

https://doi.org/10.1371/journal.pone.0253776.g005

between the treatments (p>0.7). Milk yield (kg) was maintained during the five weeks of treatments, with no difference between treatments (P = 0.1). Additionally, the treatments did not affect ECM (P = 0.3), fat content (P = 0.2), protein content (P = 0.4), or lactose content (P = 0.6).

Melatonin and IGF-1. Plasma melatonin was higher during dim night light than during daylight (P<0.001) (Fig 8). At 2230h, melatonin was significantly higher (p<0.05) for cows exposed to Blue or Red light during the day (27.7±1.7 pg/ml vs. 28.3±1.7 pg/ml) than cows exposed to White-Blue light (17.6±1.7 pg/ml), and tended to be higher (p<0.1) than in cows exposed to White light (19.2±1.7 pg/ml). The highest melatonin levels were detected at 0400h (P<0.001) in all treatments (28.6±1.2 pg/ml), when no difference was found between treatments (p>0.8). At 1600h, the lowest melatonin level (P<0.001) was detected in all treatments (8.2±0.5 pg/ml). No significant difference between the light treatments (p >0.8) was observed at 1600h or at 0830h.

Plasma IGF-1 concentration was higher at 2230 h than at 0830 h for cows in the Blue treatment (148.8±10.4 ng/mL vs. 129±10.2 ng/mL) p = 0.0002) (Fig 9). No difference was observed within the other treatments. Including all treatments, IGF-1 concentration was lowest (P<0.001) at 0830 h (143.4±5.1 ng/mL and highest (P<0.001) at 2230 h (150.5±5.2 ng/mL. No correlation was observed between melatonin and IGF-1 concentrations within 24 hours.

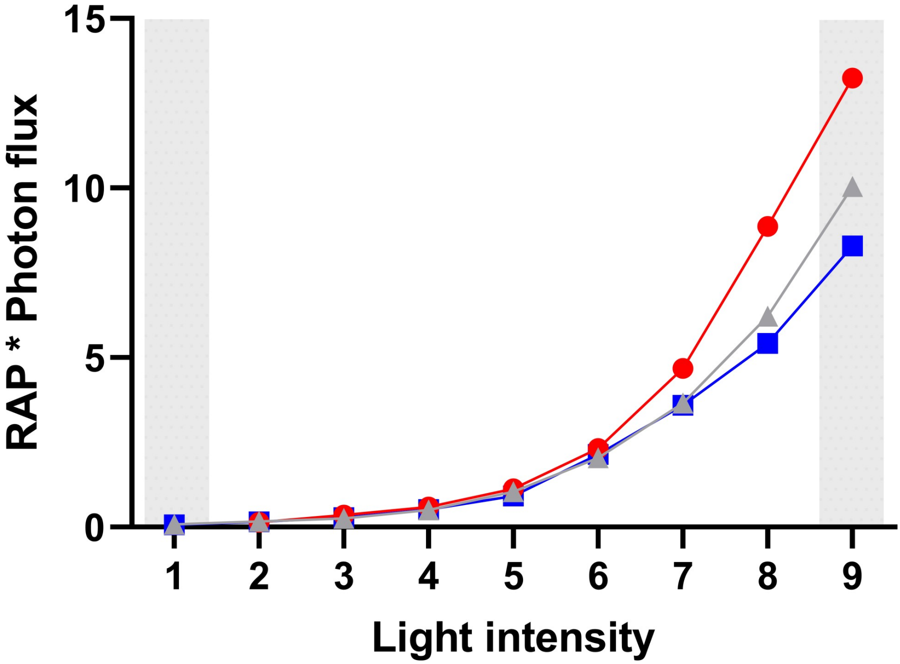

Fig 6. Relative number of photons reaching the retina when different light-emitting diode (LED) lights were employed. Grey area indicates dim night light treatment and daylight treatment in Experiment II.

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Discussion

Red light, regardless of intensity, had no significant effect on pupil size, with the RAP value obtained under red light being similar to that obtained under the dim night light ($White_1$) conditions. In contrast, bright blue and bright white lights constricted the pupils effectively, contradicting our hypothesis (i). This difference in efficacy between short and long light wavelengths is well-established in some other diurnal mammals, including humans [32]. Pupillary constriction is largely mediated through ipRGCs, but these light-sensitive ganglion cells also receive input from retinal cones and rods [13, 33]. However, cone inputs contribute less than a minute to pupillary constriction when steady-state levels of light are used, whereas rods may contribute longer, but only at light levels below saturation of the rod response [24]. In our experiments, steady levels of light were maintained for several minutes, implying no or very little cone input. Additionally, most of the light intensities tested were clearly above, the mesopic range of the bovine retina, suggesting that rod input was low. Peak absorption of melanopsin has been shown to be approximately 480 nm in other species [34–36]. The blue and white lights used in our study (the latter containing a substantial amount of short to medium wavelengths) were therefore strong stimuli for melanopsin-based photoreception, whereas red light was barely absorbed by melanopsin. Under low light intensities, our results showed no difference in pupillary size between the light colors', which indicates that our results on

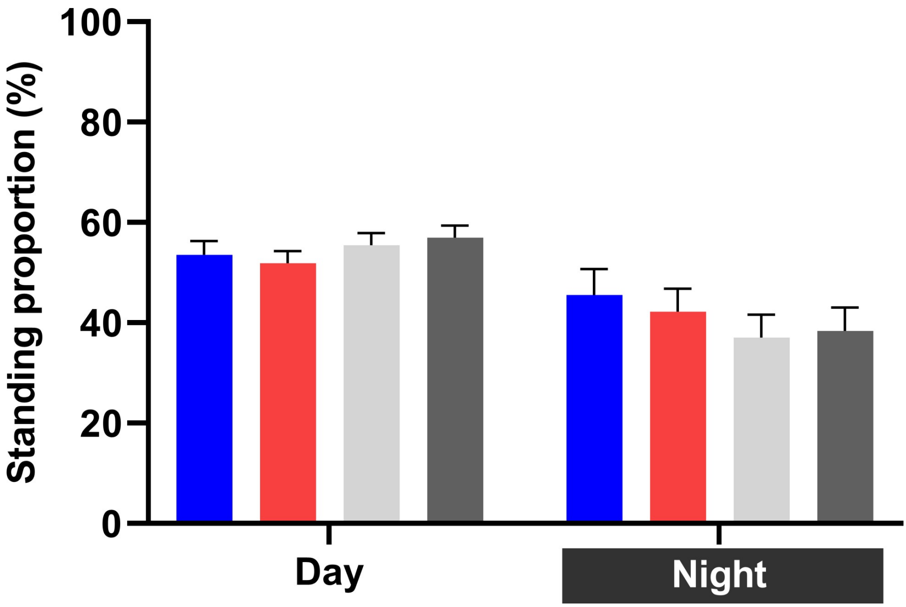

Fig 7. Treatment least squares means (LSM) for activity. Standing proportion per treatmeant, and in daytime (16 h) and night-time (8 h).

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pupillary constriction were not affected by environmental factors, e.g., stress [37]. This led us to conclude that the pupillary responses to the different lights in our experiments were mainly melanopsin-driven.

The longer resting time observed during the dark hours corresponds with results reported by Suarez-Trujillo *et al.* [38]. The lack of differences in activity between treatments may be an effect of the tie-stall system used, which restricts activity per se compared with loose housing. We did not detect any specific patterns in the activity data, e.g., whether all cows were standing or lying down at the same time, contradicting our hypothesis (ii). However, changes in activity as a result of spectrally different lighting regimes may be more apparent in a loose housing system, an issue which warrants further investigation.

Table 3. Least squares mean (LSM) ± standard error of: Milk yield, energy-corrected milk (ECM), milk composition, dry matter intake (DMI), and energy balance for cows exposed to the Blue, Red, White and White-Blue light treatments.

Variable	Blue	Red	White	White-Blue	P-value
Milk yield (kg)	32.3 ± 1.4	28.5 ± 1.4	32.2 ± 1.4	32.2 ± 1.4	0.16
ECM (kg)	33.2 ± 1.4	31.3 ± 1.3	33.7 ± 1.3	34.9 ± 1.4	0.32
Milk fat (%)	4.2 ± 0.2	4.7 ± 0.2	4.2 ± 0.2	4.6 ± 0.2	0.20
Milk crude protein (%)	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	0.42
Milk lactose (%)	4.4 ± 0.05	4.5 ± 0.05	4.4 ± 0.05	4.4 ± 0.05	0.61
DMI (kg)	24.8 ± 0.9	25.4 ± 0.9	25.4 ± 0.9	26.5 ± 0.9	0.57
Energy balance (%)	99.7 ± 2.6	97.4 ± 2.6	95.3 ± 2.6	95.5 ± 2.6	0.67

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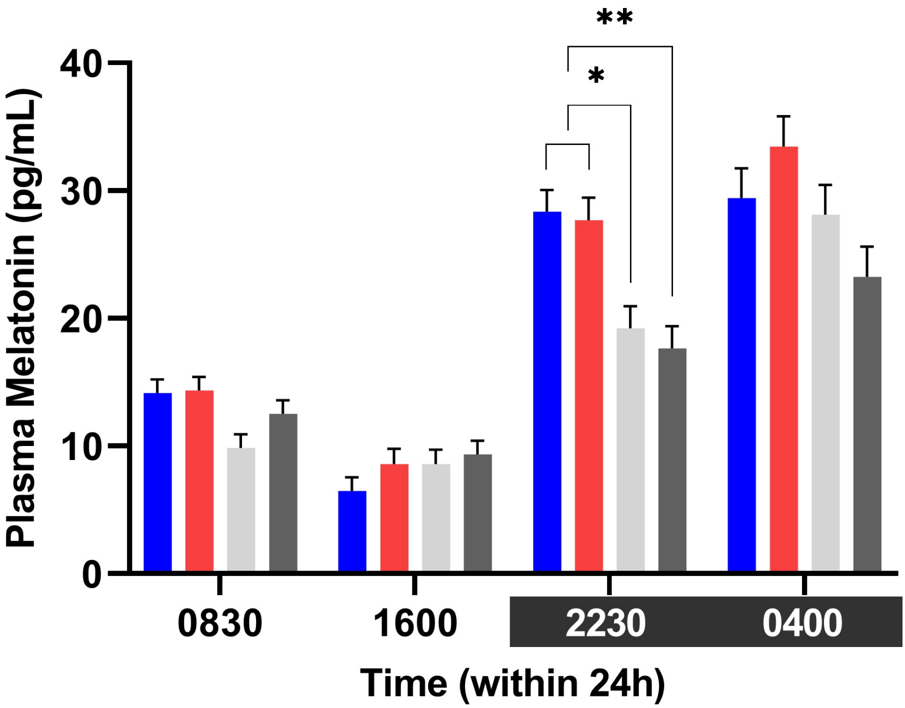

Fig 8. Treatment least squares mean (LSM) of plasma melatonin within 24 hours. Cows exposed to the Blue and Red light treatments had higher plasma melatonin than cows exposed to White light (* $p < 0.1$) and White-blue light (** $p < 0.05$).

https://doi.org/10.1371/journal.pone.0253776.g008

The diurnal rhythm in melatonin concentrations observed here, with the highest concentrations during the dark period, confirms previous findings in dairy cows [38–40] and younger cattle [22, 41–45]. The melatonin concentration increased rapidly on switching to a low light intensity, which is consistent with previous results [22, 42]. The peak melatonin level was found in the second set of samples after onset of darkness (after 7 hours in the dark), confirming results in several other studies [41, 43, 45, 46]. Interestingly, the Red and Blue light treatments caused a more rapid increase in melatonin after the onset of darkness than the White and White-Blue light treatments, contradicting our hypothesis (iii). This could be a period-treatment confounding effect, or cessation of the intense red and blue lights may have elicited more rapid secretion of melatonin. The White-Blue treatment ($White_9$ light for 10 h, $Blue_9$ light for 6 h) did not cause such a rapid increase in melatonin at night as seen with the Blue treatment ($Blue_9$ light for 16 h). Thus, the shorter exposure to blue light before the dim night light in the White-Blue treatment may not have been sufficient for a rapid response in melatonin secretion. The highest melatonin levels were obtained after the long-wavelength Red daylight treatment, although the levels were not significantly different from those in the Blue and White treatments. Elsabagh *et al.* [22] found that two hours of dim yellow LED light increased melatonin concentration faster than two hours of dim short-wave blue LED light treatment,

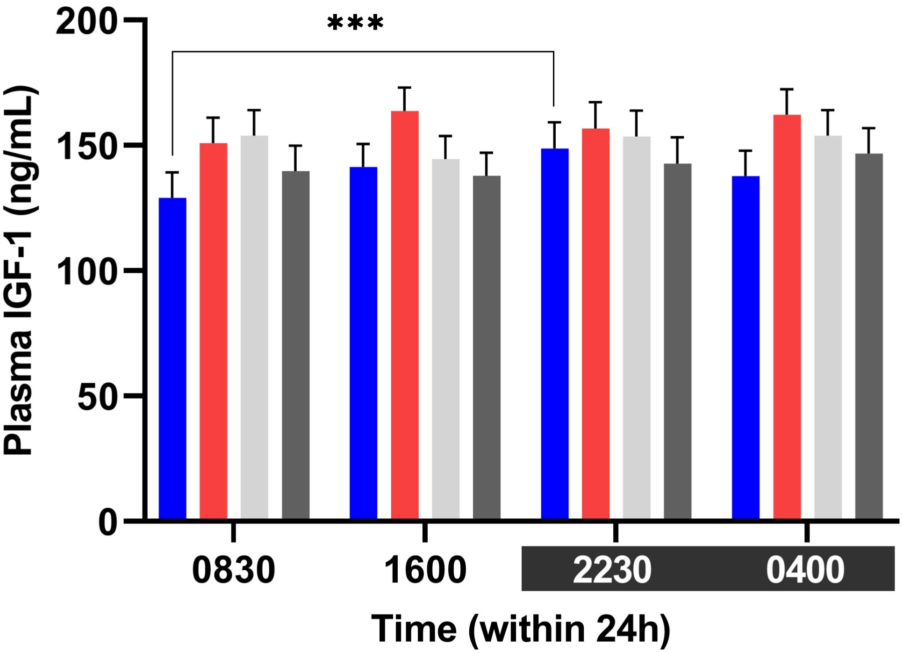

Fig 9. Treatment least squares mean (LSM) of plasma IGF-1 within 24 hours. Cows exposed to the Blue light treatment had higher plasma IGF-1 concentration at 2230 h than at 0830 h (*** $p < 0.001$).

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which suggests that dimming light after exposure to longer wavelengths is at least as effective in replenishing plasma melatonin levels as when short-wavelength light has been employed during the day. However, the light intensities used by Elsabagh *et al.* [22] were similar to our light intensity 2 and only 8-week-old calves were studied, which makes comparison with our results more difficult.

Throughout the study period in experiment II, DMI and milk yield were maintained in all treatments. Although, milk yield can be expected to decline post peak lactation [47]. Since both DMI and milk yield were maintained it suggests that LED light regardless of color stimulated a more persistent lactation. However, earlier studies have showed that a LDPP increased milk yield when compared to NDPP [3]. In our study, the maintained milk yield might be an effect of the LDPP, the effect could also be a result of the maintained DMI and the positive energy balance. Despite no effect of treatments on DMI, actual nutrient intake is unknown and may have been moderately affected by actual intake proportions of forages and concentrates. However, daily visual inspection indicated that concentrate intake was complete, and thus, confounding from this factor is unlikely. To give the concentrate in a separate bowl might be preferable, though it was not manageable in this barn due to the construction of the head fronts. In addition, when the cows moved into the Light lab, there was a change in both their environment and their milking system, from an automatic quarter milking system to a cluster milking system. A Light lab with the automatic quarter milking system used in standard management of cows in the herd could have helped to study the impact of the lighting conditions alone. No effects on milk composition caused by the prolonged photoperiod were observed, which is in agreement with previous studies [3, 46].

Cows in the Blue treatment in experiment II showed a tendency for a diurnal pattern in plasma IGF-1, though none of the other treatments indicated a diurnal pattern in IGF-1. This corresponds with earlier findings in one study [44] but not in others [3, 48]. Our results showed no correlation between plasma levels of melatonin and IGF-1 throughout the 24 hours when samples were taken. Muthuramalingam *et al.* [44] discovered a tendency for a negative correlation between IGF-1 and melatonin during night-time. However, other factors not measured in the present study may also have caused variation in circulating IGF-1, and some of these factors may have influenced plasma levels more than the light treatment. Negative energy balance is one factor that causes a decrease in circulating IGF-1 [49, 50], and often arises close to the onset of lactation [51]. Negative energy balance can explain findings that the number of days in milk, counted from the onset of lactation, and IGF-1 are positively correlated [52, 53]. In addition, IGF-1 plays an important role during pregnancy, in gonadotropin-induced folliculogenesis [50], meaning that days in pregnancy can affect the dynamics of circulating IGF-1. All cows in experiment II were pregnant, within the range of post peak lactation and prior to month 7 of pregnancy. In a previous study on cows treated with LDPP, Dahl *et al.* [3] observed increased concentrations of IGF-1 that were independent of changes in growth hormone and IGF-binding-proteins-2 and -3. A later study by Kendall *et al.* [48] showed increased concentrations of IGF-1 in LDPP calves, regardless of nutritional status. The IGF-1 concentrations reported in the literature differ markedly [3, 44, 48, 49, 52, 53], possibly due to the factors mentioned above and/or the method of analysis used in the laboratory. Our plasma IGF-1 results are similar to those obtained in a pilot study performed by Ferneborg *et al.* [54] on the same herd and with the same method of analysis. However, the number of animals in the present study was insufficient to give the statistical power needed to detect differences below 25 ng/ml.

It is interesting that melatonin and activity levels, two parameters related to diurnal rhythm, were essentially similar regardless of daylight regime. The light-driven circadian oscillator (process C) is required for partitioning sleep during the day-night cycle, whereas prolonged periods of wakefulness increase the propensity to sleep (homeostatic mechanism or process S) (see Borbely *et al.* [55] for review). In experiment II, we used a period of acclimatization before feed and milk data were sampled. Sampling for melatonin and IGF-1 analyses, and activity measurements, were made at the end of each trial period (lighting regime). Hence, we believe that our data mainly reflect the effect of the different daylight regimes on the light-driven circadian oscillator.

Both short and medium wavelengths, which are easily absorbed by ipRGCs and short-wavelength (blue) cones, had a similar effect to long-wavelength (red) light, although red light is unlikely to be absorbed by melanopsin, at least to any substantial degree. We do not believe that the higher RAP we observed for Red daylight could compensate for the poor absorption by ipRGCs. It has been shown in transgenic mice that both the rod-cone and melanopsin-driven pathways are required for normal entrainment of the circadian rhythm, and thereby the sleep cycle [8]. Therefore, it is more likely that the sleep cycle in cows under red light conditions is driven by medium- to long-wavelength cone input to ipRGCs, whereas blue and white daylight can affect both cone types in the bovine retina, as well as the melanopsin-pathway directly. Thus, we suggest that the retinal circuitry conveying light signals to the circadian oscillator in the cow shares basic features with that of both mouse and human.

In conclusion, our results show that LED fixtures emitting red light driving the ipRGCs indirectly via ML-cones, blue light stimulating both S-cones and ipRGCs directly and a mixture of wavelengths (white light) exert similar effects on milk yield and activity in dairy cows.

Furthermore, the feed intake required is not significantly different between light treatments. This suggests that the spectral composition of LED lighting in a barn is secondary to duration and intensity. Thus, the choice of spectral composition better be based on other preferences, such as visual comfort for barn staff and suitable lighting for surveillance systems. However, long-term effects of LED lighting with different spectral compositions on production parameters, as well as activity and sleep patterns in dairy cows in loose housing, warrant further investigation.

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Author Contributions

Conceptualization: Emma Ternman, Sabine Ferneborg, Johan Lindqvist, Björn Ekesten, Sigrid Agenäs.

Data curation: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg.

Formal analysis: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg, Björn Ekesten, Sigrid Agenäs.

Funding acquisition: Emma Ternman, Sabine Ferneborg, Daniel Bånkestad, Johan Lindqvist, Björn Ekesten, Sigrid Agenäs.

Investigation: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg, Björn Ekesten, Sigrid Agenäs.

Methodology: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg, Daniel Bånkestad, Johan Lindqvist, Björn Ekesten, Sigrid Agenäs.

Project administration: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg, Björn Ekesten.

Supervision: Emma Ternman, Björn Ekesten, Sigrid Agenäs.

Writing – original draft: Sofia Lindkvist.

Writing – review & editing: Sofia Lindkvist, Emma Ternman, Sabine Ferneborg, Daniel Bånkestad, Johan Lindqvist, Björn Ekesten, Sigrid Agenäs.

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In this thesis, the role of light environment and impact of light intensity, spectral composition and uniformity on dairy cows, was investigated. The results show that the color of light is of less importance for maintaining milk production, cows can navigate an obstacle course unhindered in darkness and confirmed that light intensity affects production of melatonin, important for the circadian rhythm. Light environments can be more objectively described using multiple measuring methods, preferably not only methods based on human vision.

Sofia Lindkvist received her doctoral education at the Department of Animal Nutrition and Management, Swedish University of Agricultural Sciences (SLU), and obtained her Master of Science degree in Agriculture (Animal Science) at the same university.

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