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– cows' walking behavior in dim light environments

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Abstract

Swedish dairy cows should have access to dim light at night according to the animal welfare act, but there are no further recommendations stated for dim light management regarding light intensity, light color, or light distribution. This study aimed to examine the effects of five dim light intensities, in red and white light color, and in even and uneven light distribution on cows' walking behavior in an obstacle course. The study was performed during four weeks and a change-over design was applied. Twelve cows were assigned to walk through an obstacle course in 14 light treatments including two control treatments. Five different light intensities, in red and white light, and in evenly and unevenly distributed light were tested. Time taken for the cows to pass through, and number of steps during the obstacle course were recorded. Speed, stepping rate, and step length was calculated. In addition, behavioral observations of the cows walking through the obstacle course were performed. Results showed that cows walked slower and took fewer steps per second in red, unevenly distributed light in medium intensity compared with control light ($p<0.05$) and red, evenly distributed light in low intensity ($p<0.005$). Cows' walking behavior did not differ in the darkest light treatment compared to the other light treatments ($p>0.1$). Light treatment did not affect the observed behaviors ($P>0.3$). In conclusion, the results indicate that cows can navigate in dark environments without supplementary light and in red light environments. Red, unevenly distributed light in medium intensity interfered with cows' walking behavior in the obstacle course. Further research is desirable to investigate dim light effects on cows' walking behavior in a loose housing system.

Keywords: night light, illumination, locomotion, activity, intensity, color, distribution, obstacle course.

Sammanfattning

Svenska mjölkkor ska ha tillgång till nattbelysning enligt den svenska djurskyddslagen, men det finns inga ytterligare rekommendationer kring hur nattbelysningen bör vara utformad gällande ljusintensitet, ljusfärg eller ljusarmaturernas fördelning. Studiens syfte var att undersöka hur fem olika ljusintensiteter, i vitt och rött ljus samt jämn och ojämn ljusfördelning påverkar kors gångbeteende i en hinderbana. Studien genomfördes i en change-over design under fyra veckor där totalt tolv kor gick genom en hinderbana i fjorton ljusbehandlingar inklusive två kontrollbehandlingar. Fem olika ljusintensiteter, i rött och vitt ljus, samt jämnt och ojämnt fördelat ljus testades i försöket. Tidtagning och antal steg mättes när korna gick genom hinderbanan. Kornas hastighet, steg per sekund och steglängd beräknades. Kornas beteende observerades och noterades när de passerade genom hinderbanan. Studiens resultat visade att korna gick långsammare och tog färre steg per sekund i rött, ojämnt fördelat ljus i medel intensitet jämfört med kontrolljuset ($p < 0{,}05$) och rött, jämnt fördelat ljus i låg intensitet ($p < 0{,}005$). Kors gångbeteende förändrades inte i mörker i jämförelse med de andra ljusbehandlingarna ($p > 0{,}1$). Kornas observerade beteende påverkades inte av någon ljusbehandling ($P > 0{,}3$). Slutsatsen var att kor verkar kunna navigera genom en hinderbana i mörka miljöer utan belysning samt i rött ljus. Rött, ojämnt fördelat ljus i medel intensitet påverkade kors gångbeteende i hinderbanan. Slutligen behövs mer forskning för att undersöka hur kors gångbeteende påverkas av nattbelysningens utformning i lösdrifter.

Nyckelord: nattbelysning, mjölkko, aktivitet, rörelsemönster, intensitet, färg, fördelning, hinderbana.

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Abbreviations

AMS	Automatic milking system
CL	Control light treatment
CD	Control dark light treatment
LSM	Least square mean
M/L-cone	Middle-to-long wavelength cone
RE_{low2}	Red even distributed light treatment in low2 intensity
RE_{med}	Red even distributed light treatment in medium intensity
RE_{high}	Red even distributed light treatment in high intensity
RU_{low2}	Red uneven distributed light treatment in low2 intensity
RU_{med}	Red uneven distributed light treatment in medium intensity
RU_{high}	Red uneven distributed light treatment in high intensity
S-cone	Short wavelength cone
SEM	Standard error of the mean
WE_{low1}	White, even distributed light treatment in low1 intensity
WE_{low2}	White, even distributed light treatment in low2 intensity
WE_{med}	White, even distributed light treatment in medium intensity
WU_{low2}	White uneven distributed light treatment in low2 intensity
WU_{med}	White uneven distributed light treatment in medium intensity
WU_{high}	White uneven distributed light treatment in high intensity

1. Introduction

Vision is one of the most important senses for cows to obtain information about their environment (Phillips 2002). Cows are prey animals that, in an evolutionary aspect, have relied on their vision to protect themselves from predators or other fearful situations. In addition, cows are grazing animals that historically have been outside during both daytime and night, requiring good vision in various types of light situations. It is not known how cows perceive different light environments, but it is proven that cows' vision differs from humans (Phillips 2002).

Artificial light management is a tool that is used to influence milk production and physiology in dairy cows (Dahl et al. 2012). According to the animal welfare act (SJVFS 2019:18), Swedish dairy cows should have access to daylight inlet and dim light at night. Lighting should be designed to support cows' circadian rhythm and behavior without causing discomfort. The animal welfare act has, however, no further recommendations of dim light management at night regarding light intensity, light color, or light distribution (SJVFS 2019:18). In Sweden, most cows are held in a loose housing system with an automatic milking system (AMS) or conventional milking (Jordbruksverket 2019). In AMS, the cow traffic is of great importance for production efficiency (Jacobs & Siegford 2012). The cow traffic is the flow and movement of cows between areas of feeding, resting, and milking. A milking robot in AMS is an expensive investment (Jacobs & Siegford 2012), that preferably should be in action both day and night, in a cost-efficient aspect. To support production at night, the cows need to be able to see and navigate in the barn, and the use of lighting at night might increase cows' confidence in walking (Phillips et al. 2000). There is, however, limited research on how dim light at night should be managed to support cows' walking behavior. Some producers use bright lights during 24 hours per day to ensure that the cows can navigate in the barn and to encourage feeding and milking also at night (Pettersson & Wiktorsson 2004). It has however been confirmed that cows need a dark period of approximately 8 hours per day to support circadian rhythm and animal welfare (Chamberlain, 2018; Dahl et al. 2012; Modi et al. 2017), but which light intensity is suitable during the dark hours in not yet known. Further, some commercial companies promote red light at night with the explanation that cows do not perceive red light and that it does not disturb circadian rhythm but enable staff to observe the animals (reviewed by Lindkvist et al. 2021). This has been questioned by researchers, and it has been shown that cattle can distinguish red light from blue and green lights (Phillips & Lomas 2001; Gilbert & Arave 1986). How cows perceive the red light, and if cows change their walking behavior in red dim light is still however unknown. To enhance cow movement and cow traffic in a loose housing system, the distribution of light is also important (Grandin 1997a). If lighting is unevenly distributed at night, for example if the night luminaires are placed far apart, shadows might appear which have been shown interrupting walking in cows (Grandin 1997a). Despite this, some Swedish dairy farmers provide an unevenly distributed light at night in the barn, presumably due to its simplicity. Lightings in dairy barns are commonly supplied by fluorescent lamps and metal halide lights (Harner & Zulovich 2014). The interest in using light-emitting diodes (LEDs) increases since they are more energy-efficient, have a longer lifetime, and are dimmable (Harner & Zulovich 2014; Pattison et al. 2018; Son et al. 2020). Ten years ago, lightings in dairy barns in Sweden accounted for about 10% of the total energy use of the production (Hörndahl et al. 2012). It is of great importance to design the lighting in an appropriate way to reduce the use of energy and increase the profitability and environmental sustainability of the production.

This study aimed to examine the effects of five dim light intensities, in red and white light color, and in even and uneven light distribution on cows' walking behavior in an obstacle course. The research questions were i) Do the light treatments tested in this study affect cows' walking behavior in an obstacle course? and ii) Do cows modify their walking behavior through an obstacle course in a dark environment without supplementary light compared with a brighter light environment? The hypothesis was that cows' walking behavior differ in low light intensities compared with higher light intensities and that cows' walking behavior is different in red light compared with white light. In addition, walking behavior might be different in even light distribution compared to uneven light distribution.

2. Literature review

2.1. Cow vision

Visible light is electromagnetic radiation that can be perceived by the eye (Starby 2006) and appears in wavelengths of around 400-700 nm for most mammals (Sjaastad et al. 2016). Cows are prey animals with close to 360° vision to protect themselves from predators (Sjaastad et al. 2016). Cows have 25° - 50° binocular vision, where both eyes are focused on the same object, providing three-dimensional images (Grandin 1980). Consequently, cows have poor depth vision in comparison with predators with eyes placed closer together (Grandin 1980). Cow vision is dependent on three types of photoreceptor cells called intrinsically photosensitive retinal ganglion cells (ipRGCs), rods and cones, located in the retina of the eye (Tosini et al. 2016). ipRGCs have multiple functions, including regulating circadian rhythm and adjusting pupil size (Tosini et al. 2016). Rods are stimulated in dim light environments and give vision in greyscale (Gilbert & Arave 1986). In contrast, cones are activated in brighter lights and provide color vision (Gilbert & Arave 1986). Cows have dichromatic vision, including short-wavelength cones (S-cones) and middle-to-long wavelength (M/L) cones (Jacobs et al. 1998). S-cones are most sensitive to light wavelengths of 455 nm, which corresponds to blue color, and M/L-cones have a peak sensitivity to light wavelengths of 554 nm, which is perceived as green. However, cones seem to be susceptible to wavelengths ranging around 410 – 650 nm (Jacobs et al. 1998), which corresponds colors from violet to orange (Starby 2006). Hence, it is generally believed that cows cannot see red color. According to Hörndahl (2012), red light is perceived as grey with low light intensity by cows. In contrast to cows, humans have trichromatic vision including cones with peak sensitivities around 430 nm, 530 nm, and 560 nm, which corresponds to blue, green, and red color (Brown & Wald 1963). Therefore, humans appear to have a more extensive color vision in comparison to cows.

Cows seem to have good vision in low light intensities due to their large eyes, thereby receiving more light compared to a smaller eye (Phillips et al. 2000). In nature, dim light vision might be beneficial to search for feed and for protection at night (Phillips 2002). The cow eye has a proportion of 5-6 rods per cone at the periphery of the retina which is comparable to the proportion of rods and cones in the human eye of 20:1 (Dannenmann 1985; Gilbert & Arave 1986; Phillips et al. 2000). However, cows, unlike humans, have an extra light-reflecting layer in the eye, called tapetum lucidum (Phillips et al. 2000). This mechanism will increase the eyes' sensitivity to light but also reduce visual acuity (Sjaastad et al. 2016). In addition to the vision, cows also rely on their hearing and smelling to obtain information of their surroundings, and these senses are particularly important when the sight is limited (Moran & Doyle 2015).

2.2. Cow behavior

Domesticated and wild cows are distinct flock animals and lives in groups (Jensen 2017). In nature, cows form groups together with other cows and their calves while bulls establish separate groups. Cows perform daily behaviors including feeding, drinking, ruminating, socializing, standing, walking, resting, self-grooming and more (Jensen 2017; Phillips 2018). Motivation for performing a certain behavior is caused by specific stimuli (Dannenmann et al. 1985). A behavior can be caused by instinct, sensory perception, or by experience (Moran & Doyle 2015). Behaviors caused by instinct are performed by intuition and do not require learning, such as breathing and grazing. Sensory perception is caused by stimuli like a smell, taste, feeling, or a noise in the environment, thereby motivating a certain behavior. For example, if a loud noise appears in the surrounding, cows might feel scared and walk the other way. Behaviors caused by experience have been taught earlier in life, such as walking to the feed table when feed is delivered (Moran & Doyle 2015).

Behaviors can be observed by direct or indirect methods (Xue & Henderson 2006). With a direct method, animal behavior is observed and registered in real time. Video recording is an indirect method to observe animals and perform behavioral studies, making it possible to observe data several times (Xue & Henderson 2006). Although, video recordings only show a small part of reality and there might be difficulties in capturing the full context (Haidet et al. 2009). Results of behavioral studies might differ among observers regardless of observation method (Xue & Henderson 2006). Therefore, it is advantageous if the same observer evaluates all material (Haidet et al. 2009). To increase reliability of behavioral studies, the observer needs to be trained to identify specific behaviors. Additionally, video recordings can be blinded to unable observers knowing which treatment is present. Blinding video material increases the independent evaluation of the material by the observer and reduces the risk of biased results (Haidet et al. 2009).

2.3. Light intensity

Light intensity varies naturally during day and night (Dahl et al. 2012). Natural daylight differs in light intensity, from 1000 lux a cloudy day to 100 000 lux in direct sunlight (Chamberlain 2018). Moonlight can provide 0.05 – 0.3 lux (Chamberlain 2018). In Sweden, light intensities in dairy barns of 100 – 150 lux at daytime and 5 lux at night is recommended (SIS-TS 37:2012). However, recommendations of night light intensity differ between countries (Phillips et al. 2000). In Germany, France, and Switzerland, recommended light intensities in dairy barns are 20, 30 and 60 – 120 lux, respectively (Phillips et al. 2000).

The amount of light emitted from a light source per second is defined as luminous flux and is measured in lumen (Starby 2006). The luminous flux per surface unit is determining illuminance, which is one of the most common ways to measure light intensity (Starby 2006). Illuminance is presented in lux ($lumen/m^2$) (ASAE 2006) and is developed for the human spectra and white light (Hörndahl et al. 2012). Another method to determine light intensity is measuring the number of light particles, photons, emitted from a luminaire, which can be quantified as photon flux density (PFD) (Starby 2006). PFD is defined as *µmol * s-1 * m-2 * nm-1* and can be measured with a spectrophotometer. Besides the number of photons, the spectrophotometer also defines the wavelengths of the photons (Starby 2006).

2.3.1. Circadian rhythm

Fluctuations in light intensity are important to maintain circadian rhythm in cows (Dahl et al. 2012). Cows' circadian rhythm regulates behavior and physiology during a period of approximately 24-hours (Jensen 2017; Piccione et al. 2011). It has been found that cows are more active at daytime than at night (Piccione et al. 2011). Cows' circadian rhythm is partly regulated by the concentration of the hormone melatonin, which is related to light and darkness (Dahl et al. 2012). In darkness, melatonin is produced in the pineal gland. Melatonin is essential to fixate circadian rhythm to around 24 hours. When light is perceived by photoreceptors in the eye, signals are transferred through nerves to the pineal gland. Consequently, melatonin synthesis and secretion will be inhibited, thereby stimulating wakefulness (Dahl et al. 2012). Piccione et al. (2011) showed that cows generally are feeding and moving at daytime while resting at night, which is closely related to the light-dark cycle. However, in loose housing systems, cows' circadian rhythm might be interfered, and cows tend to spread out feeding and lying behaviors at day and night since the delivery of fresh feed and milking might occur at any time of the day. The natural photoperiod ranged between 12-15 hours in the study, but it is not stated what lighting was used indoor, if any (Piccione et al. 2011).

2.4. Locomotion and walking behavior

In nature, cows move to perform activities essential for life, such as searching for feed and escaping fearful situations (Piccione et al. 2011). Locomotion is voluntary movements that involve the whole body (Phillips 2002). Cow locomotion depends on multiple factors, such as health state and circadian rhythm as well as environmental aspects like lighting and flooring (Anderson 2003). Feed access, social- and climatic factors also affect locomotion (Phillips 2002). Walking is the most important part of locomotion for cows. Cows' walking behavior includes for example number of steps, speed, step length and other behaviors that cows perform while walking. When taking a step, the leg is lifted from the ground and swung forward, and then placed back on the ground (Phillips 2002). For example, the rear leg is lifted and swung to the place where the front hoof just has been lifted from (Anderson 2003). Cows prefer to walk at a speed of 0.6 – 1 m/s, which is the comfort zone where there is a small difference in the energetic efficiency of walking (Phillips 2002). If speed is exceeding, cows energetically benefit to trot or gallop. Cows walk with a step length of about 1.2 m if the ground or floor has good friction (Phillips et al. 2000).

2.4.1. Dim light environments

In dim light, the cow pupil is transformed from an oval to a round shape and diluted to absorb maximal number of photons (Lindkvist et al. 2021; Rehkämper et al. 2000). In humans, pupillary dilution and dark adaptation are nearly finished in one minute and complete in less than 10 minutes (Wagman & Gullberg 1942). During dark adaptation, rods produce a pigment called rhodopsin, which enables sight in darker environments (Finley 1959). Furthermore, when cells are exposed to bright light, rhodopsin pigment is disaggregated. The procedure of adaptation to a brighter light environment is very quick and human cones are fully adapted in less than 10 minutes (Asakawa et al. 2019).

Phillips et al. (2000) discovered, in two experiments, that cows adapt their walking behavior in surroundings with low light intensities. In the first experiment, cows were walking in a passageway with supplementary light (259 lux) and in darkness (0 lux). The passageway led from the milking unit back to the herd, and the floor in the passageway was partially covered in slurry. The luminary consisted of a single halogen lamp placed in the middle of the passage. The number of steps and the time to walk through the passageway were recorded. Furthermore, speed (m/s), stepping rate (step/s), and step length (m) were calculated. Results showed that cows increased stepping rate in darkness but reduced step length and maintained speed. The researchers concluded that taking more steps per second was the safest way to maintain speed in the dark. In the second experiment, cows were walking in a passageway in different light intensities of 0, 0.7, 4.3, 32, 119, and 250 lux. The light was provided by incandescent lamps in the lower intensities (0.7 and 4.3 lux) and fluorescent tubes for the remaining intensities. Cows were acclimatized to the new light environment for at least 20 minutes before each test. The same walking behavior parameters were recorded as in experiment 1. In this experiment, the floor in the passageway had good friction without slurry coverage. The results showed that cows increased their speed in 0 lux, and that speed was lowest at 32 lux. The researchers discussed that cows increased speed in the dark because they might have felt frightened and wished to reunite with the herd. A lower speed was assumed to be related to composure. Cows changed their walking behavior by taking more vertical steps by changing the angle of limb joints in lower light intensities. Furthermore, it is believed that 250 lux might have caused glaring, affecting cows' walking behavior negatively. In conclusion, Phillips et al. (2000) recommended a dim light of 32-119 lux to support cow walking.

Dannenmann et al. (1985) examined the effects of light intensity on calf behavior. Light intensities of 2, 20, 100, and 130 lux were tested. The luminaries consisted of neon tubes. Calves rested more frequently and for a longer time in 2 lux compared with 20, 100 and 130 lux. Additionally, feeding behavior lasted a longer time in 100 and 130 lux, and calves were more actively play-fighting in 100 and 130 lux compared to 2 and 20 lux (Dannenmann et al. 1985).

Hjalmarsson et al. (2014) did not find an effect of light intensity at night on number of passages through selection gates in automatic milking systems. The study was performed at three Swedish farms with a Feed-First system, meaning that cows feed before entering the milking unit to access the resting area. Night light intensities of 11, 33, and 74 lux were compared. The cows were attending at least one treatment, but all cows did not attend every light treatment. The light treatments were performed in a predetermined cross-over design on the farms. Light intensity was measured with a lux meter. Unfortunately, it is not mentioned which type of luminaires that was used in the barns. The results showed that cows passed gates more frequently during daytime than at night. However, total number of gate passages per 24 hours did not differ between night light intensities. Reducing night light intensity to 11 lux did not affect cows' movements between sections in the barn compared to higher dim light intensities. However, milk yield was lower in 11 lux. Hjalmarsson et al. (2014) concluded that supplementary lights at night might be closer related to production parameters than to animal welfare.

Pettersson & Wiktorsson (2004) found no effects on cows' preference to rest in a stable with full lighting (200 lux) compared to guide light (5 – 7 lux). The resting area was divided into two equal parts, one with full lighting at night and the other half with guide light at night. After three weeks, lights were reversed between resting areas. Results showed no differences in cows' preference to rest in a specific area on a herd level, since cows were equally distributed in both parts of the resting area at night. However, there were some individual preferences shown among cows, meaning that some cows preferred to rest in guide light while other cows showed a preference for resting in full lighting.

2.4.2. Dark passageways

It has been proved that cows prefer lit areas (Phillips & Weiguo 1990; Phillips & Morris 2001; Stookey & Watts 2007), which might be due to better visual contact between cows, contributing to social behavior and hierarchism, but also prevention of injuries in lighted environments (Penev et al. 2014). Also, cows avoid dark passages (Phillips et al. 2000; Phillips & Morris 2001; Stookey & Watts 2007), which may appear if the lighting is insufficient. Phillips & Morris (2001) investigated non-lactating dairy cows' preference of walking through a lit or dark passageway. The passageway was designed as a Y-shape, with one passage at start which later was divided into two passageways. Both passageways led back to the herd. At first, cows were trained to walk through the passage that provided the greatest feed reward. Later, one of the two arms in the passageway was lit up (22 lux) and the other was dark (0.03 lux). The results showed that cows strongly avoided the dark passageway, even though they were offered a feed reward only in the dark passage. A similar study was conducted by Stookey & Watts (2007) that examined cows' behavior walking through a Y-shape passageway. One arm of the passageway was lit up while the other one was dark. The result showed that 42 of 49 cows (86%) chose to walk through the lighted arm of the Y-passageway. This suggests that cows prefer lighted passages (Stookey & Watts 2007).

2.4.3. Light distribution

The American Society of Agricultural and Biological Engineers defines light distribution as the ratio between the maximum and minimum light intensity in a specific area (ASAE 2006). Lightings in barns should be evenly distributed to enhance cow movement (Grandin 1997a). If lights are unevenly distributed in barns, the risk of shadows or dark passages appearing is increased, which might interfere with cows' walking (Grandin 1997a). Thereby, it is important to avoid shadowing and dark passages in barns to maintain a well-functioning cow traffic (Chamberlain 2018; Phillips et al. 2000). Despite the importance of evenly distributed illumination, dim lights at night are unevenly distributed among some Swedish dairy farms, according to a survey in a master thesis by Jakobsson (2016). The farms provided night light either by using separate luminaries, or by turning off sections of the lights used during daytime. Other farms used all luminaires for daylight on, but at low power to provide dim light at night (Jakobsson 2016).

2.5. Light color

White light consists of a combination of wavelengths over the visible spectrum (Cho et al. 2017). Consequently, white light is a mixture of blue, green, and red light. White LED light has peak radiation of 450 – 470 nm, corresponding to blue light (Tosini et al. 2016). It appears that short wavelengths are activating ipRGCs to a greater extent than long wavelengths (Fonken & Nelson 2014). Lindkvist et al. (2021) found that cow pupil size was not affected by increasing intensity of red colored light. This suggest that ipRGCs, that adjusts pupil size, were not sensitive to the increased intensity of light with long wavelengths. In contrast, the pupil constricted with increasing light intensity of blue and white light. This result indicates that there might be a difference in how cows perceive red colored lights compared with white and blue colored light (Lindkvist et al. 2021). Several studies have discovered that cattle can discriminate red color from blue and green colors (Phillips & Lomas 2001; Gilbert & Arave 1986). Phillips & Lomas (2001) investigated the ability of calves to distinguish red, green, and blue color of light. Eleven calves were attending the study, in which three of them were trained to select for blue color, four calves were assigned to distinguish green, and four calves to select red light. Calves were positioned in front of two chambers with one colored light in each. If calves were entering the right chamber, they got a concentrate reward. The researchers discovered that calves were, after training, able to distinguish red light from green and blue light. However, calves had difficulties distinguishing between green and blue light, presumably due to their dichromatic vision. Phillips & Lomas (2001) did also investigate the effects of light color on calves' movements. Calves' number of movements per minute were highest in red light compared with blue and green light, suggesting that calves were more active in red light.

Gilbert & Arave (1986) conducted a similar study in which they compared the ability of heifers to differentiate between red, blue, and green colors. Heifers were taught to discriminate between colors by entering one of two chambers with red, green, or blue light color. If the heifer chose to enter the correct chamber and pushed a plate, a feed reward was given. In similarity to Phillips & Lomas (2001), the researchers found that cattle can distinguish red from green and blue. Additionally, cattle showed some uncertainty to separate blue from green color. However, the results differed among the heifers since some animals were more confident to discriminate colors than others (Gilbert & Arave 1986).

Lindkvist et al. (2021) did not find an effect of light color on tied-up cows' duration- and frequency of standing and lying behavior in white and red light. Lying and standing behavior differed between daytime and night, but not between light colors (Lindkvist et al. 2021).

2.6. Fearful situations

Fear is an emotion that causes stress in cows (Grandin 1997a). Fear can be induced by stimuli such as novelty, isolation from other cows (Grandin 1997a; Jensen 2017) and possibly also lighting (Son et al. 2020). In the wild, novelty is often an indication of danger (Grandin 1997a). Examples of novelty in dairy production are foreign objects or shadows appearing by inadequate lighting. In fearful situations, cows adjust their behavior (Grandin 1997a). Moran & Doyle (2015) discuss that cows defecate and urinate more frequently if scared. Cows might also adjust their walking behavior and refuse to pass the fearful stimuli (Moran & Doyle 2015). In a non-peer reviewed article by Grandin (1997b), the researcher discuss that cows might stop moving and stand still in front of an obstacle or a shadow created by the lighting (Grandin 1997b). Since cows have inadequate depth vision, cows might also lower their head to obtain a clearer sight of foreign objects close to the ground (Grandin 1997b). In many cases, cows will avoid danger and walk the other way rather than risk getting injured (Moran & Doyle 2015). Another sign of fear and stress is vocalizing (Grandin 2001). In the study, vocalization observations were recorded in 48 slaughterhouses in North America and Australia. In 11 slaughterhouses, a problem of cows vocalizing was found, which was assumed to relate to inadequate lighting like dark passages (Grandin 2001). Adjustments in light management are one of the most common ways to improve animal welfare and enhance cow movement in slaughterhouses (Grandin 2006).

In a study by Phillips & Lomas (2001), calves reached their handler faster in a fearful situation in red light compared to blue and green light. This suggests that calves were calmer in blue and green light than in red light (Phillips & Lomas 2001). Son et al. (2020) recorded stress levels as plasma cortisol levels in dairy cows subjected to yellow, white, and blue colored light. Cows in blue light had higher plasma cortisol levels than cows in white and yellow light. The yellow light resulted in the lowest plasma cortisol levels. Cortisol is a metabolic hormone that is secreted in stressful situations (Son et al. 2020) to release energy for a flight-flight response. Cortisol is generally involved in metabolism of nutrients and the concentration also increases after feeding in mammals (Sjaastad et al. 2016). In contrast to Phillips & Lomas (2001) did Son et al. (2020) not recommend blue light to lactating cows due to the increased plasma cortisol levels.

3. Materials and methods

3.1. Animals and housing

The trial was conducted during four weeks at the Swedish Livestock Research Centre in Uppsala, Sweden. The experiment was performed during 2021-06-07 – 2021-06-18 and 2021-06-28 – 2021-07-09. Twelve non-lactating cows were included in the study and blocked according to days since dry off (25-46), days before predicted calving date (25-35), breed (Swedish red (n=9) and Swedish Holstein (n=3)), and parity (1-4). All cows were checked for good leg and claw health. Complete information of the cows is stated in Appendix 1. A change-over design was applied by dividing the cows into four groups with three cows in each group. One group of cows were housed for one week. Unfortunately, one cow in group 2 had to be removed from the trial since leg problems were discovered during the second day of tests. All animal handling was approved by the Uppsala Ethics Committee for Animal Research, Uppsala, Sweden (reference no. 5.8.19-06780/2020).

The cows were housed individually in three pens (9 m^2 each) from Monday morning until Friday afternoon. The pens were placed in line with each other, enabling a cow to interact with the cow in the pen next to them. A detailed view of the barn is shown in Appendix 2. The barn consisted of a temporary herding aisle and an area used for the obstacle course constructed for the purpose of this study. The barn had no windows for daylight inlet and a controlled light environment were created in the barn with the artificial light. Roughage (95% silage + 5% straw) were fed *ad libitum* and there was one automatic water trough in each pen. The nutrient content of the silage is given in Table 1. Pens and water troughs were cleaned three times a day, at 07:00, 12:00, and 16:00. Wood shavings were used as litter in the pens and was refilled three times a day.

Table 1. Nutritional content of the silage

Nutrient	Value	Unit
Dry matter (DM)	351	g/kg
Ash	87	g/kg DM
Crude protein	149	g/kg DM
Neutral detergent fiber (NDF)	433	g/kg DM
Metabolizable energy	10.6	MJ ME/ kg DM

3.2. Light treatments

The lighting in the barn was supplied by 18 LED lamps [LX602G, Heliospectra AB, Göteborg, Sweden] placed in two rows at a height of 2.9 meters over the floor. The intensity, color, and distribution of the light were managed manually through a computer. In the study, 14 light treatments including two control treatments were tested (table 2 and figure 1). Control light (CL) was a white light that resembles the spectral distribution of sunlight and is close to comparable with the control light in Phillips et al. (2000). In the other control treatment, control dark (CD), all lights were turned off with exception for the emergency lights close to the pens. Five different light intensities were tested (dark, low1, low2, med, and high), as well as red and white lights. The red light treatments contained most of its photons (97.5 %) within the wavelengths of red light. In the study, even and uneven light distribution were also tested. The evenly distributed light treatments included all 18 LED lamps turned on, and the uneven light treatments included only three of 18 luminaires on with equal distance in the barn.

CL was used as barn lighting at 05:00 – 09:00 and 16:00 – 21:00. At night, 21:00-05:00, light treatment WE_{low1} was used as lighting. The light fixtures were cleaned from dust and checked for temperature weekly. Before each test, light was measured on eight points in the center of the obstacle course at a height of 1.25 m, which corresponds to the approximately height of cows' eyes. The lighting was checked to ensure that the light settings were correct. Light measurements were performed with a spectrometer [PAR200 Quantum Spectrometer, UPRTEK, Europe, Aachen, Germany], which quantifies light in lux and PFD. The light in the obstacle course was also measured per square meter by several measurement tools to make a detailed description of the light environment in the stable. In this master's thesis, only the spectrometer data will be used to explain the light environment. During the trial, another master's student was focusing on the light measurements, and the full description of the light environment can be found in her master's thesis (C.F.).

*Table 2. Description of the light treatments and ordinary barn light measured in the obstacle course, including mean, minimum, and maximum light intensity in photon flux density (PFD) (µmol * s-1 * m-2 * nm-1) and lux (lumen/m2). Measurable points describe how many measuring points out of 48 that the spectrometer was capable to measure. CL= control light, CD= control darkness, W= white light, R= red light, E= evenly distributed light, U= unevenly distributed light.*

	Photon flux density			Lux			Measurable
Light	Mean	Min	Max	Mean	Min	Max	points (n=48)
CL	3.60	3.19	4.48	209.16	181.33	264.71	48
CD	0.00	0.00	0.00	0.00	0.00	0.00	0
WE_{low1}	0.12	0.11	0.15	8.51	7.46	10.76	48
WE_{low2}	0.28	0.25	0.34	18.97	16.56	23.88	48
WE_{med}	0.73	0.65	0.89	50.21	43.47	63.19	48
WU_{low2}	0.13	0.07	0.22	8.55	4.78	14.83	12
WU_{med}	0.27	0.04	0.59	18.65	2.47	38.91	15
WU_{high}	0.61	0.02	2.85	35.31	1.05	161.55	36
RE_{low2}	0.28	0.23	0.38	3.46	2.92	4.72	48
RE_{med}	0.77	0.55	6.65	7.86	0.52	10.99	48
RE_{high}	3.23	2.62	4.47	39.52	32.99	54.85	48
RU_{low2}	0.20	0.19	0.20	2.49	2.45	2.52	2
RU_{med}	0.30	0.20	0.46	3.66	2.54	5.56	11
RU_{high}	1.42	0.97	2.28	17.27	11.78	27.59	12
Barn	6.35	4.54	8.01	444.89	318.01	560.79	48

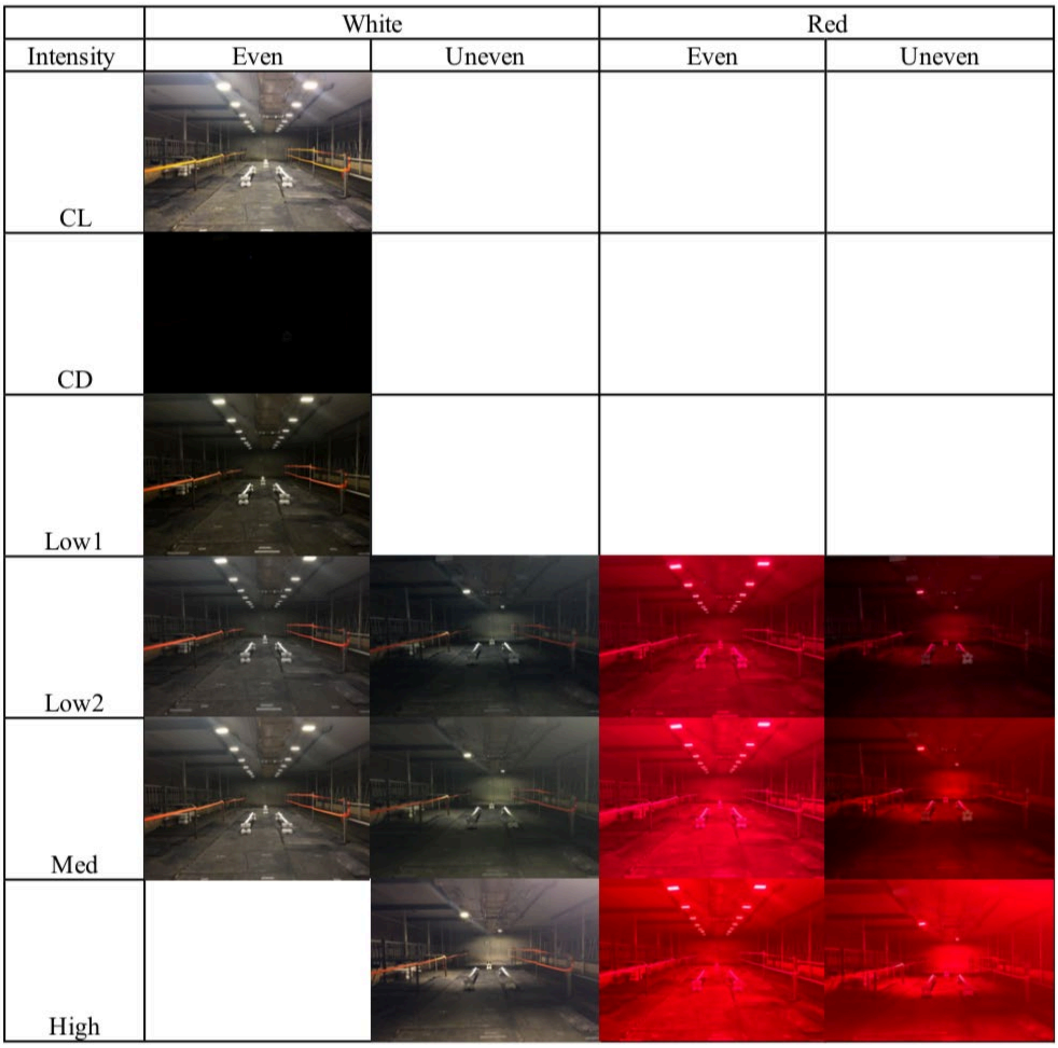

Figure 1: Picture scheme of the light treatments. CL = control light, CD= control darkness.

3.3. Experimental design

Three cows arrived to the barn each Monday morning weekly during the experiment. The cows were acclimatized to the new environment for at least 5 hours before they were trained to walk through the obstacle course three times in the ordinary barn lighting. During training sessions, a fixed obstacle course design was used, and no measurements were taken. The trial days (Tuesday – Friday) included seven tests per cow. The cows were provided teaser feed at the end of each test to encourage them to walk through the obstacle course. An example of a test day schedule for light treatments is given in table 3. Each test day started with running the cows, one at a time, through the obstacle course in CL followed by CD. Thereafter, three additional light treatments were tested per day. The order of these three light treatments was randomized, but blocked by intensity, meaning that they were going from darker to brighter light to ensure light adaptation. Cows were acclimatized at least 10 minutes before the test when changing from a darker to a brighter lighting. If the lights were changed to a darker intensity, the acclimatization period was at least 45 minutes. At the end of each trial day, CD and CL were tested again. The reason for testing CD also at afternoon was to reduce the effect of going from darker to brighter light. CL was tested at the end of each day to compare with the morning results and investigate if there were any differences between the tests, such as the cows getting tired of walking in the obstacle course after a day full of tests. CL and CD were also tested each day to see if there were any effects of day of week, for example to investigate if cows responded differently in the beginning of the week compared with the end.

Table 3. An example of the order of light treatments that were tested for one day

Light treatment schedule, one day
CL
CD
WE_{low2}
RU_{med}
WU_{high}
CD
CL

The obstacle course was 14.5 m. long and 3.75 m. wide. There were in total 28 different obstacle course designs (Appendix 3). One cow went through the same obstacle course design once. When all cows completed the obstacle course in a light treatment, the obstacle course design was rebuilt. The obstacles that were used were white, plastic show jumping obstacles commonly used for horses. The floor in the obstacle course was covered with rubber mats.

3.3.1. Recordings

During the tests, the time (s) and steps (no.) through the obstacle course were measured. The time through the obstacle course was measured with a stopwatch and number of steps were counted manually during tests. The recordings of time and number of steps started when a cow entered the obstacle course with its right rear leg and stopped when the cow had passed the stop line with the same leg. Further, speed (m/s), stepping rate (steps/s) and step length (m) were calculated. Speed was determined by time through the obstacle course and its length. Stepping rate was calculated with number of steps and time through the obstacle course. Step length was determined by number of steps and length of the obstacle course. The tests were recorded by infrared video cameras, a mobile phone [iPhone 8, Apple Inc, Cupertino, California, United States] and a thermal camera [NightLux JSA IR-

635, Dualoptic, Reute, Germany]. Cow behavior was observed indirectly using the video recordings. The behaviors observed are stated in table 4 and it was registered if a behavior was performed or not in a test. The video recordings were blinded, meaning that the name of the light treatment was unknown to the observer (KS.) with the intention to avoid biased results. The order of behavioral observations was randomized with exception for group. The video material from the infrared cameras and the thermo camera appeared similar regardless of light treatment, meaning that light treatment could not be determined by those video materials. The video recordings from the mobile phone camera were of higher quality and were showing if the lighting was white or red, and what distribution of light was used, but it was difficult to identify the intensity by the video recordings. In the experiment, the other master student measured the cows' heart- and respiratory rate before and after each test.

Table 4. Ethogram containing the behaviors that were observed and their definition

Behavior	Definition	Unit
Step	Right hind leg is lifted up in the air and put down on the floor	No.
Interaction obstacle	The cow interacts with obstacle by sniffing, licking or touching with any part of the body without knocking down the obstacle to the floor	Yes/no
Self-grooming	The cow interacts with herself by licking or scratching with tongue or claw	Yes/no
Interaction floor	The cow interacts with the floor by sniffing or licking	Yes/no
Interaction surrounding	The cow interacts with any other surrounding by sniffing, licking, or touching with any part of the body	Yes/no
Knock-down	The cow touches an obstacle, and the obstacle falls down to the floor	Yes/no
Jump obstacle	The cow steps or jumps over an obstacle with all four legs without knocking it down	Yes/no
Stand still	The cow stands with at least three hoofs on the floor for at least five seconds	Yes/no
Slipping	The cow does a sliding movement with a leg along the floor	Yes/no
Vocalization	The cow creates a sound with her vocal cord for at least one second	Yes/no
Defecation	The cow defecated or urinated	Yes/no

3.4. Statistical analysis

The mixed procedure in SAS (SAS version 9.4, SAS Institute Inc., Cary, NC.) was used to test whether time through the obstacle course, speed, stepping rate, number of steps, and step length were affected by light treatment (CL, CD, WE_{low1}, WE_{low2}, WE_{med}, WU_{low2}, WU_{med}, WU_{high}, RE_{low2}, RE_{med}, RE_{high}, RU_{low2}, RU_{med} and RU_{high}). In all models, treatment and group (1-4) were included as fixed effects and cow nested within treatment and group as a random effect, with a first-order autoregressive structure. Accordingly, it was considered that the tests were correlated to each other, and that the correlation was stronger between tests closer in time than between tests further apart in time. 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 data was transformed using base 10 (log) and back-transformed both means and measures of variability using the delta method (Onofri et al. 2010).

Four behaviors (interaction with obstacles, standing still, interaction with floor and interaction with surroundings) occurred in every light treatment. To test the probability of those behaviors to occur, the GLIMMIX procedure of SAS was used. If probability equals 1, the behavior always occurred in a light treatment, and if probability is 0, the behavior never occurred in a light treatment. In all models, treatment and group were included as fixed effects and cow nested within treatment and group as a random effect. The effect of test day and time of day were tested.

Values presented are least squares mean (LSM) ± standard error of the mean (SEM), unless otherwise stated. Results were considered significant at $P\leq0.05$, while a trend was assumed for probabilities $0.1 > P > 0.05$. Post-hoc means separation for significant main effects was applied using Tukey-Kramer's adjustment of probability values.

4. Results

4.1. Time and number of steps

Cows spent longer time, walked slower, and had lower stepping rate in the obstacle course in light treatment RU_{med} compared to CL ($p<0.05$) and RE_{low2} ($p<0.005$) (table 4). Cows tended to spend longer time in the obstacle course and walk slower in RU_{med} compared to RE_{high} ($p=0.08$). Cows in group 2 spent shorter time, walked faster, and had higher stepping rate in obstacle course than cows in group 1 ($p<0.005$). Light treatment tended to affect number of steps ($p=0.08$) and step length ($p=0.08$). No effect of day in treatment or time of day was found.

Table 4. Back-transformed means ± back-transformed measures of variability via the delta-method (Onofri et al. 2010) for time, number of steps, speed, stepping rate, and step length in the obstacle course in different light treatments. CL= control light, CD= control darkness, W= white, R= red, E=evenly distributed, U= unevenly distributed. Back-transformed means with different superscript letters show a significant difference (p<0.05).

Light	Time (s)	Steps (no)	Speed (m/s)	Stepping rate (step/s)	Step length (m)
CL	25.8 ± 1.4^a	12.1 ± 0.2	0.56 ± 0.03^a	0.47 ± 0.02^a	1.20 ± 0.02
CD	32.5 ± 1.7	12.7 ± 0.2	0.45 ± 0.02	0.39 ± 0.02	1.14 ± 0.02
WE_{low1}	30.0 ± 2.8	12.2 ± 0.3	0.48 ± 0.04	0.41 ± 0.03	1.19 ± 0.03
WE_{low2}	33.6 ± 3.0	12.6 ± 0.3	0.43 ± 0.04	0.38 ± 0.03	1.15 ± 0.03
WE_{med}	35.5 ± 3.2	13.3 ± 0.3	0.41 ± 0.04	0.38 ± 0.03	1.09 ± 0.03
WU_{low2}	34.7 ± 3.1	13.0 ± 0.3	0.42 ± 0.04	0.37 ± 0.03	1.12 ± 0.03
WU_{med}	29.9 ± 2.8	12.5 ± 0.3	0.48 ± 0.05	0.42 ± 0.03	1.16 ± 0.03
WU_{high}	32.9 ± 3.0	13.0 ± 0.3	0.44 ± 0.04	0.40 ± 0.03	1.11 ± 0.03
RE_{low2}	19.7 ± 1.7^a	11.3 ± 0.3	0.74 ± 0.07^a	0.57 ± 0.04^a	1.29 ± 0.03
RE_{med}	28.9 ± 2.7	12.1 ± 0.3	0.50 ± 0.05	0.42 ± 0.03	1.20 ± 0.03
RE_{high}	24.6 ± 2.3	12.0 ± 0.3	0.59 ± 0.05	0.49 ± 0.04	1.20 ± 0.03
RU_{low2}	38.0 ± 3.5	13.2 ± 0.3	0.38 ± 0.04	0.35 ± 0.03	1.10 ± 0.03
RU_{med}	61.5 ± 5.6^b	14.5 ± 0.3	0.24 ± 0.02^b	0.24 ± 0.02^b	1.00 ± 0.02
RU_{high}	29.5 ± 2.7	12.3 ± 0.3	0.50 ± 0.05	0.42 ± 0.03	1.18 ± 0.03

4.2. Behavioral observations

Light treatment had no effect on cow behavior analyzed ($P>0.3$). The behaviors were numerically most frequent performed in RU_{med} (44 tests), and least behaviors were performed in RE_{low2} (14 tests) (table 5). Cows jumped obstacles during two tests in light treatment CL, RU_{low2} and WE_{low2} and no cow jumped over an obstacle in CD, WU_{low2}, RE_{low2}, RU_{med}, WU_{high} and RU_{high}. Cows knocked down obstacles during 6 tests in WU_{med} and cows never knocked down an obstacle in RE_{low2} and WU_{high}. Cows self-groomed in all light treatments but WE_{med} (fig. 13). Defecation occurred during 6 tests in RU_{med} but did never occur in RE_{low2} and WU_{high}. Cows slipped at the floor two times, once in light WE_{low2} and once in light RE_{med}. Vocalization did not occur in any tests.

Table 5. The sum of tests that one behavior was observed in per light treatment. Light treatment did not affect cow behavior (P>0.3). CL= control light, CD= control darkness, W= white light, R= red, E=evenly distributed light, U= unevenly distributed light, obst = interaction with obstacles, jump = jumping obstacles, knock = knock down, still = stand still, floor = interaction with floor, groom = self-groom, surr = interaction with surrounding, def = defecation.

Light	Obst.	Jump	Knock	Still	Groom	Floor	Surr	Def	Slip
CL	5	2	3	5	1	6	6	1	0
CD	8	0	3	5	2	6	7	2	0
WE_{low1}	5	1	1	5	2	3	5	1	0
WE_{low2}	8	2	4	6	2	6	6	5	1
WE_{med}	6	1	2	5	0	4	5	1	0
WU_{low2}	5	0	2	6	1	5	9	4	0
WU_{med}	4	1	6	6	2	5	4	1	0
WU_{high}	4	0	0	8	2	5	9	0	0
RE_{low2}	4	0	0	3	1	2	4	0	0
RE_{med}	7	1	2	7	2	6	7	1	1
RE_{high}	3	1	2	3	1	3	1	2	0
RU_{low2}	7	2	4	8	2	5	6	4	0
RU_{med}	10	0	2	8	2	9	7	6	0
RU_{high}	5	0	2	7	3	4	4	3	0

The probability of occurrence (0= never occurred, 1=always occurred) of interaction with obstacles, standing still, interaction with surroundings and interactions with floor is presented in figure 2. Cows interacted with obstacles most frequent in RU_{med} (probability 0.84) and least frequent in RE_{high} (probability 0.26). Cows stood still most frequent in RU_{med} (probability 0.73) and least frequent in RE_{low2} and RE_{high} (probability 0.22). The probability that cows interacted with the surrounding was highest in WU_{high} and WU_{low2} (probability 0.77), and lowest in

RE_{high} (0.07). Cows interacted with the floor most frequent in RU_{med} (probability 0.76) and least frequent in RE_{low2} (probability 0.15). Cows in group 2 interacted more often with the surrounding and the floor than cows in group 1 ($p<0.05$). Also, cows in group 2 stood still more frequent than cows in group 1 ($p< 0.005$) and 3 ($p<0.05$).

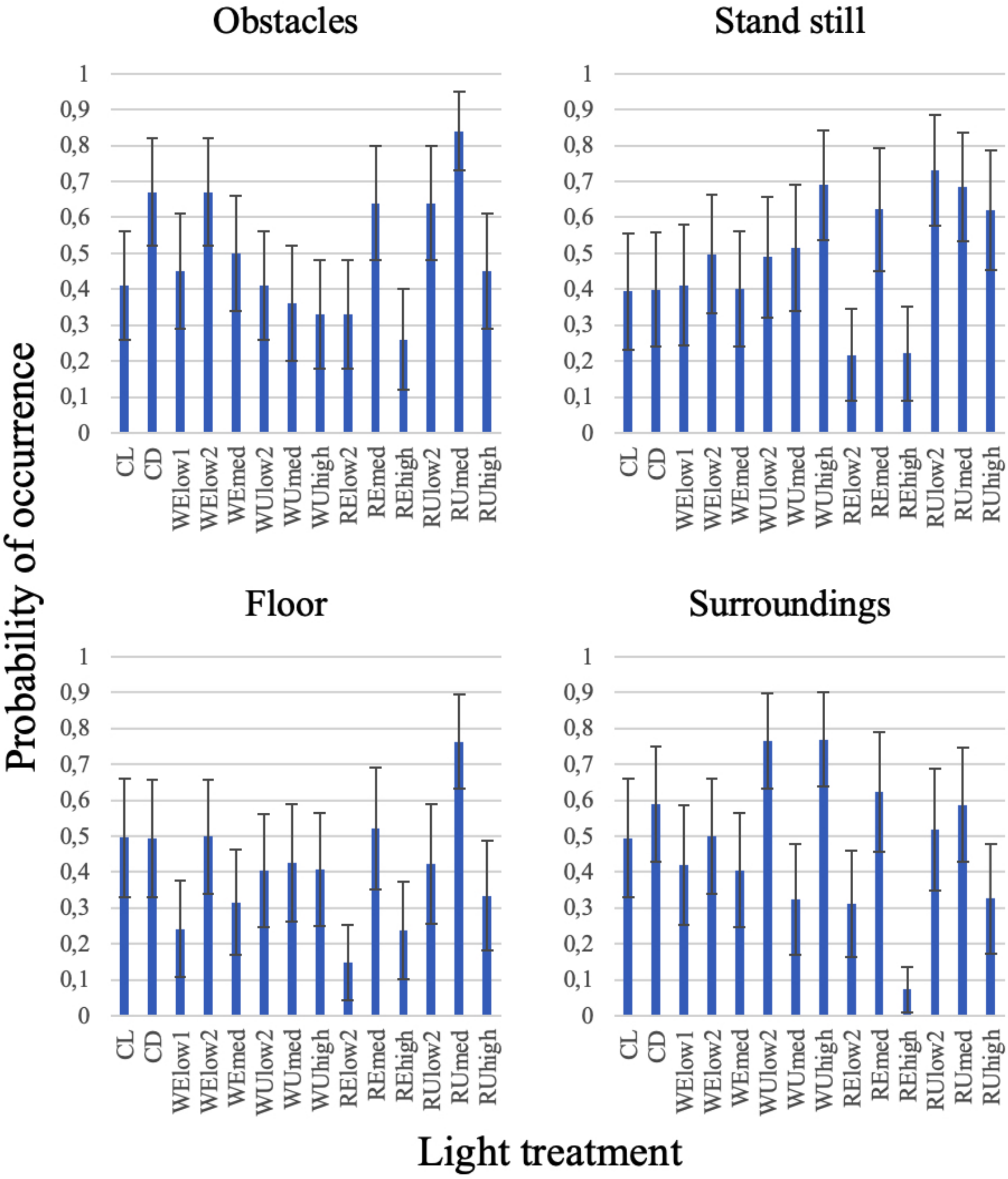

Figure 2. Least square mean ± standard error of the mean (SEM) of the probability that interaction with obstacles, stand still, interaction with floor, and interaction with surroundings occurred in the light treatments. The probability ranges from 0 (never occurred) to 1 (always occurred). CL= control light, CD= control darkness, W= white light, R= red, E=evenly distributed light, U= unevenly distributed light.

5. Discussion

The results of this study suggest that cows can navigate in dark environments without supplementary light. Contradicting the hypothesis, cows' walking behavior was not significantly different in CD compared to the other light treatments. From an evolutionary point of view, vision at night was probably beneficial since cattle are prey animals that seek protection in fearful situations (Phillips 2002). This supports our results that vision in dark environments might be possible. However, our results do not correspond with previous research by Phillips et al. (2000) that discovered that cows increase speed in the dark compared with higher light intensities. Phillips et al. (2000) conducted the study in a passageway without obstacles, leading from the milking unit back to the herd. This passageway was used in everyday routines and was a familiar environment to the cows, which might have increased cows' confidence in increasing speed. The presence of novel obstacles in an unfamiliar environment might have been one of the reasons that cows did not increase speed in CD. Cows prefer to walk with a speed of 0.6 – 1 m/s (Phillips 2002), and with a step length around 1.2 m (Phillips et al. 2000). The cows in our trial walked through the obstacle course generally slower than stated in Phillips (2002). Probably, cows reduced their speed due to the obstacles crossing their path in the obstacle course, forcing the cows to walk over or around them. Cows' step length in this study is close to the step length found in Phillips et al. (2000). In our study, the finish line of the obstacle course was placed close to the cows' pens. In contrast, the passage in Phillips et al. (2000) continued after the finish line, enabling cows to keep on walking when the test was completed, which might have encouraged the cows to accelerate their speed in the passage. Phillips et al. (2000) describe their dark treatment as 0 lux, in similarity to our study. However, CD was not equal to zero photons, although the spectrometer classified the environment as too dark to measure. Photons were intruding from outside the barn through small openings at doors, and some light was also emitted from the emergency lamps. Perhaps, that amount of light was enough for cows to navigate through the obstacle course without altering walking behavior. In a commercial dairy barn, it will never be completely dark, and light will be coming from outdoor and other light emitting devices will brighten up the barn at night. The results of this study suggest that the intensity of night lighting in barns to dairy cows could be reduced without interfering with cows' walking behavior. This would reduce the use of energy, resulting in an increased profitability and sustainability of the production. From an ethical point of view, a lowered night light would decrease the risk of disturbing cows' circadian rhythm. Night lighting might however be more important in a social aspect, such as an increased security for farm staff working in the barn at night. This study was conducted in a controlled environment that excludes several environmental factors, and the results may not be representative of commercial farms. However, our results are consistent with those of Hjalmarsson et al. (2014), not finding an effect of light intensity (11, 33, and 74 lux) at night on how cows move between feeding-, resting- and milking areas in commercial dairy farms. Unfortunately, lower intensity than 11 lux was not tested by Hjalmarsson et al. (2014), and how cows navigate without supplementary light at night in commercial dairy farms would be interesting to examine further.

In contrast to the hypothesis, no effect of light color was found on cow's walking behavior since there was no difference between light treatments with equal intensity and distribution, but different colors, such as RU_{med} and WU_{med}. This result suggests that cows possibly can navigate in red light. How cows perceive the red light is however not known. Phillips & Lomas (2001) and Gilbert & Arave (1986) found that calves were able to distinguish red light from a blue and green light. However, these kinds of studies give no information on how cattle perceive the red color, and further research is needed. Hörndahl et al. (2012) suggests that cattle see red color as grey with low light intensity, but the researchers do not explain why. To increase knowledge about color perception in cows, it would be interesting to investigate if cows can distinguish between red and grey colors with an equal amount of photon flux. If cows cannot distinguish between red and grey, this would support the statement of Hörndahl et al. (2012). Son et al. (2020) found that the cortisol concentration in blood was higher in cows subjected to light with short wavelength in comparison to light with longer wavelengths. Cortisol levels are increased also during other circumstances than fearful situations (Sjaastad et al. 2016). Therefore, the results of Son et al. (2020) might have been more reliable if plasma cortisol measurements would have been supplemented with other stress indicators, such as behavioral observations or physiological measurements like heart rate or respiratory rate. Phillips & Lomas (2001) found that cattle are more active during fearful situations in red light compared to green and blue light, which was discussed to be a response of stress. Why a certain light color, such as red colored light, would affect the physiological and behavioral response in cattle are not explained in the studies, and perhaps the response in cows is more closely related to the perceived light intensity rather than the long wavelengths per se. Further research is needed to investigate whether cows respond differently in certain light colors with equal light intensity. Vocalization can be a sign of stress (Grandin 2001). In our study, vocalization did not occur in any test, suggesting that cows were not frightened during the trial. Another sign of stress or fearful situations is an increased frequency of defecation (Moran & Doyle 2015). In this study, no clear trend could be found that cows defecated more frequently in a certain light treatment, suggesting that light did not stress the cows during the trial.

No clear effect of light distribution was found on cows' walking behavior in this study. However, the probability that cows stood still, interacted with obstacles, the floor and surroundings was numerically highest in unevenly distributed light treatments. Although not significant, this suggests that cows navigated more easily in evenly distributed light since the observed behaviors from the video recordings were fewer. These results reflect those of Grandin (1997b) who also found that cows stop and lower their head to the floor to examine shadows or obstacles closer, due to their poor depth vision. Since Grandin (1997b) is a non-review article, its reliability can be questioned. It is not stated in the article how the researchers conducted the study and how they could conclude that cows change their walking behavior in front of shadows or other obstacles. Earlier findings agree that lighting should be evenly distributed to support cows' walking behavior and cow traffic (Chamberlain 2018; Phillips et al. 2000; Phillips & Morris 2001; Stookey & Watts 2007). In addition, cows seem to avoid dark areas in the barn without supplementary lighting (Phillips & Morris 2001; Stookey & Watts 2007), and these might arise in unevenly distributed lightings (Grandin 1997a). This could be a potential problem in loose housing systems if there is insufficient lighting in passages, by the milking unit or by the feed table. If unevenly distributed light is used, the placement of luminaries is suggested to be of great importance to support cow movement. A further study with focus on placements of luminaires causing unevenly distributed light in a loose housing system is therefore suggested. However, with the increased popularity and use of LEDs as barn lighting, the lights can be dimmed (Harner & Zulovich 2014; Pattison et al. 2018; Son et al. 2020). Due to LEDs' many advantages, this type of light will probably be used to a greater extent in the future among Swedish dairy farmers, which will increase the possibility to use an evenly distributed light at night.

A light environment using red colored light in combination with unevenly distributed light should probably be avoided in barns to dairy cows since the cows in this study reduced speed and stepping rate in the RUmed treatment. On farms with a loose housing system, this might risk altering cow traffic if the flow between feeding-, resting- and milking area is disturbed by cows walking slowly, or hesitates in front of narrow passages, thereby blocking the paths for other cows. This might cause fewer visits to the feed table or the milking unit, perhaps altering production. Interestingly, cows' speed and stepping rate differed in RU_{med} compared with RE_{low2}, which both are red lights. However, the light distribution and intensity were different between the light treatments. To the human eye, it seemed like uneven light distribution in medium light intensity created more distinct shadows compared with low light intensity. Possibly, the more distinct shadows in medium intensity compared to low2 intensity influenced cows' speed and stepping rate. Rods and cones in the retina of an eye are activated at different light intensities (Gilbert & Arave 1986). Possibly, the light intensity in RU_{med} was in an intermediate position, too bright for activating rods, but not bright enough to activate cones. This mechanism might have limited cows' sight in the light treatment and changed the locomotion. However, it is still unknown what light intensity that activates different types of photoreceptors, and further research is needed.

A significant group effect was found in the study. However, this result might be misguiding since one cow from group 2 was taken out of the trial. Consequently, fewer tests were completed in group 2 compared with the other groups, meaning that the data were unevenly distributed among groups. Additionally, it was clear during the tests that some cows were very careful walking through the obstacle course, while other cows rushed through to achieve the feed reward. Some cows performed many behaviors repeatedly, while other cows seldom performed the observed behaviors. Cows' personalities should therefore be considered performing behavioral studies.

In this study, no effects of obstacle course designs on cows' walking behavior could be found, suggesting that the obstacle course designs were of equal difficulty. Although, the obstacle course designs had a different number of obstacles, and in different directions. Using different obstacle course designs will reduce the learning effect of cows since the obstacle course is unfamiliar, which probably was advantageous. On the other hand, using the same obstacle course design throughout the whole trial would minimize environmental variation between tests.

The light was quantified at 48 points in the obstacle course. However, the measurement instrument was not sensitive enough to measure all points in every light treatment. These points are excluded from the mean light intensity. Consequently, the mean light intensity is probably over-estimated in the light treatments with non-measurable points. The spectrometer had difficulties measuring light in CD and the unevenly distributed lights. The illuminance (lux) differs between white and red light in the same intensity level. Most likely, the spectrometer that was used as a lux meter was not as sensitive to red light and therefore the illuminance was lower in red light than white light. However, PFD is more equal between the light colors at an equal light intensity level. This suggests that PFD is a more suitable way to quantify and compare light intensity between light colors.

Cows are distinct flock animals that naturally live in groups (Jensen 2017). Consequently, cattle might feel stressed when isolated and are motivated to reunite with the herd in such situations. In this study, this flock effect was used to motivate the cows to go through the obstacle course back to the other cows. However, the barn used in this study was quite small and the cows' sense of feeling isolated was probably minimal. Phillips et al. (2000) used the same strategy for motivation since cows were walking through a passageway that led back to the herd. Because of cows' strong motivation to be together, this effect must be considered doing research. Perhaps, the flock effect was one of reasons that no differences among light treatments were shown, and that cows were more motivated reuniting with their friends than care for a special lighting. Petterson & Wiktorsson (2004) did not see an effect of cows' preference in resting in full light (200 lux) in comparison with dim light (5-7 lux). Possibly, cows' motivation of resting with certain other cows is more important than light management. The same effect is probably present in all studies that include cows or other flock animals, and the effect might be difficult to eliminate.

Several dairy farmers with AMS want to maximize the use of milking robots with a bright light at night (Pettersson & Wiktorsson 2004). However, it has been shown that circadian rhythm is disturbed in cows constantly exposed to bright light (Dahl et al. 2012). The interest in increasing cow movements at night might therefore be questioned in an ethical aspect. Lighting that causes adverse effects on animal welfare should not be used in commercial dairy barns. Further research and development of dim light managements with an ethical focus is suggested, such as investigating lightings' effects on melatonin- and stress hormone response in dairy cows.

The behavior data of jumping obstacles, knock-down, self-grooming, defecation and slipping could not be analyzed statistically due to few observations. Perhaps differences exist between these behaviors among light treatments that could not be concluded in this study. If the number of cows included in the study, and the number of tests included in the study would increase, the possibility of the behaviors occurring would also increase. However, it is not ensured that the increased power of the study would guarantee that these behaviors would occur.

6. Conclusion

Cows' walking behavior in the obstacle course did not differ between the control dark treatment and the other light treatments, indicating that cows can navigate in dark environments without supplementary light. Walking behavior was not different in red light color compared to white light color, suggesting that cows also can navigate through an obstacle course in red light. No clear effects of light distribution on cows' walking behavior were found. Red, unevenly distributed light in medium intensity was the only light treatment that affected cows' walking behavior in the obstacle course since cows walked slow and took few steps per second. Further research is desirable to investigate dim light effects on cows' walking behavior in a loose housing system.

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Popular science summary

Most Swedish dairy cows are housed in a loose housing system where the cows move, more or less, freely between the areas in the barn. According to Swedish legislation, dairy cows should have access to natural daylight at daytime and dim light at night, but there are no further recommendations of night light management regarding intensity, color, or distribution. Previous research has shown that cows need a dark period per day to rest, but which light intensity that is suitable at night is not yet known. Some commercial companies thereby sell red night lightings with the argument that red light does not disturb the cows' diurnal rhythm but enables the staff to observe them at night. Earlier research has however questioned this statement and discovered that cows possibly perceive the red light in some way, but probably not as humans do. Some dairy producers supply night lighting with single lamps, or lamps placed far from each other, which creates a lighting that is brighter in some areas of the barn, while darker in other areas. This uneven distribution of light can create shadows or dark passages in the barn, which have been shown to be avoided by cows, thereby interfering with cow traffic and cows' walking behavior.

This study aimed to investigate five low light intensities, in red and white color, and in even and uneven distribution on cows' walking behavior walking in an obstacle course. Twelve cows were assigned to walk through the obstacle course in 14 light treatments of varying light intensity (dark, low1, low2, medium, and high), light color (red and white), and light distribution (even and uneven). The time taken for the cows to walk through the obstacle course and number of steps were recorded. Cow behavior walking through the obstacle course was also observed. The results showed that cows reduced their speed in red, unevenly distributed light compared with the control light. Walking behavior did not change in darkness compared to the other light treatments. The observed cow behavior was not affected by lighting.

In conclusion, it seems like cows can navigate through an obstacle course in dark environments without supplementary lighting, and in red light environments. Red, unevenly distributed light in medium intensity was the only lighting that interfered with cows' walking behavior in the obstacle course. Further research is needed to investigate dim light effects on cows' walking behavior in a loose housing system.

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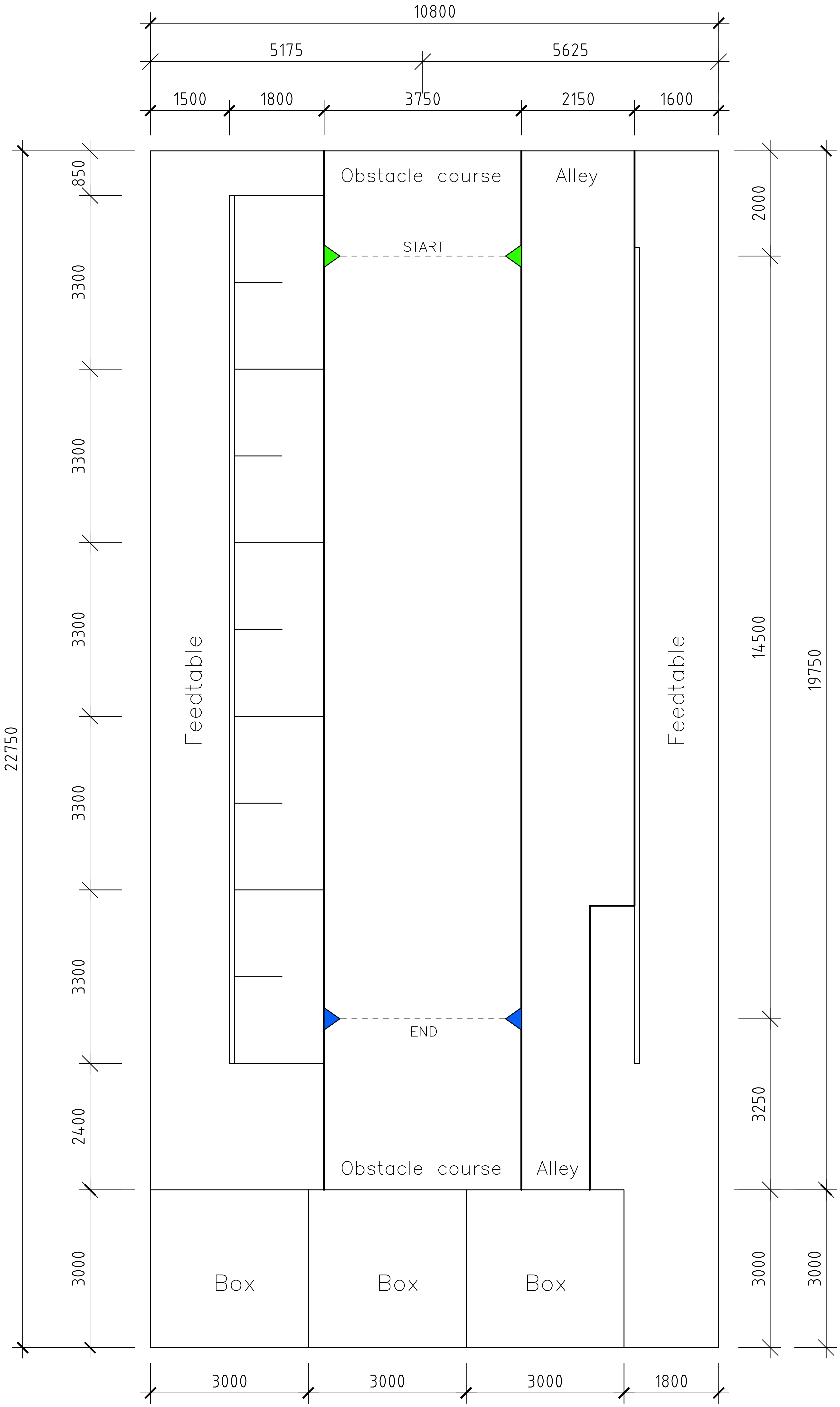
Appendix

Appendix 1: Information about the cows

*Information about the cows that were used in the experiment, including group, cow ID, breed, parity number, days until predicted calving date, days since dry off, pen and birth date. SH = Swedish Holstein, SRB = Swedish Red Breed. *Cow 541 was taken out of trial due to leg problems during the second day of tests.*

Group	Cow ID	Breed	Parity	Days to calving	Days since dry off	Pen	Birth date
1	690	SH	3	25	33	1	2016-03-19
1	869	SH	2	27	39	2	2017-07-07
1	583	SRB	4	28	46	3	2015-06-29
2	541*	SRB	4	28	39	1	2015-02-03
2	880	SRB	2	27	41	2	2017-07-27
2	2090	SRB	1	29	29	3	2018-07-21
3	2058	SRB	1	29	34	1	2018-05-21
3	894	SRB	2	34	34	2	2017-08-26
3	912	SRB	2	31	34	3	2017-10-18
4	2100	SRB	1	29	32	1	2018-08-10
4	642	SRB	4	35	29	2	2015-12-06
4	558	SH	4	31	25	3	2015-03-26

Appendix 2: Illustration of the barn



Appendix 3: Obstacle course designs

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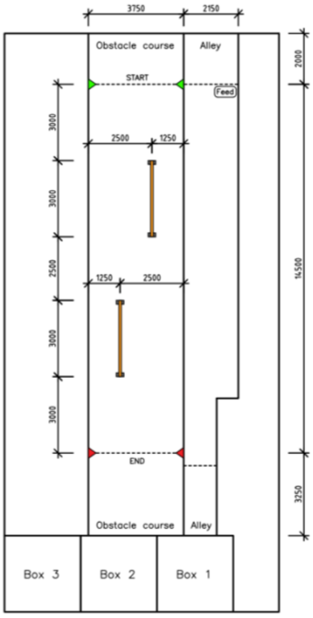

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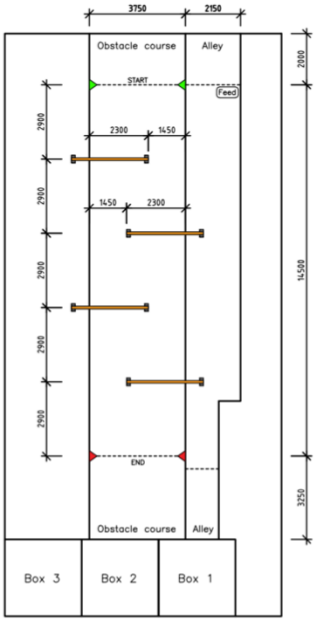

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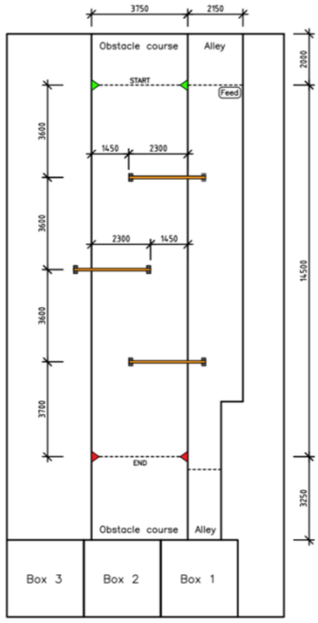

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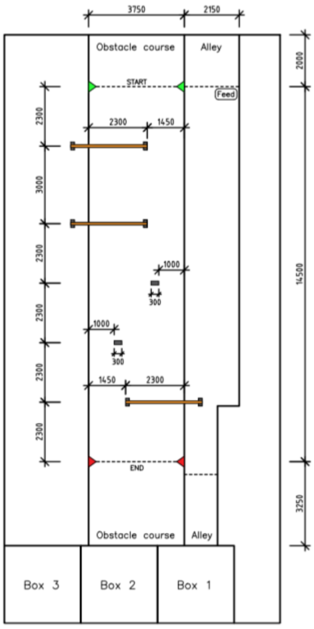

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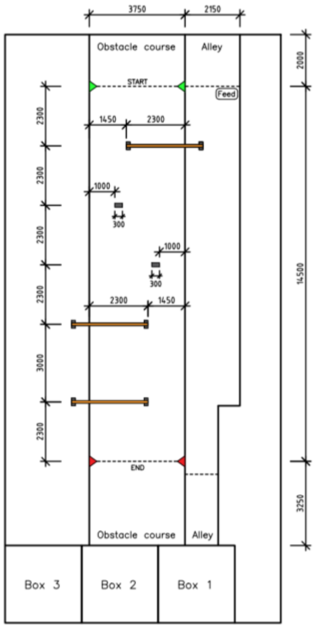

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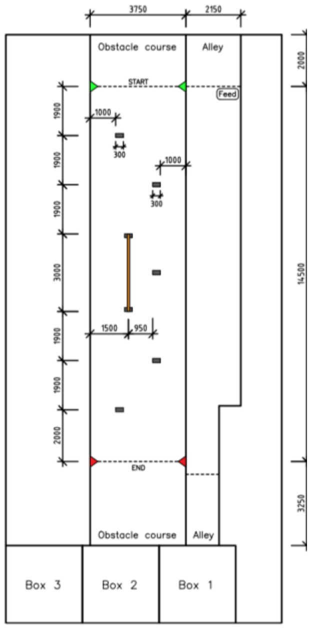

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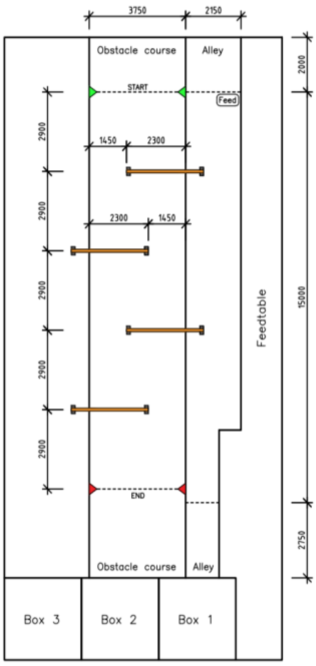

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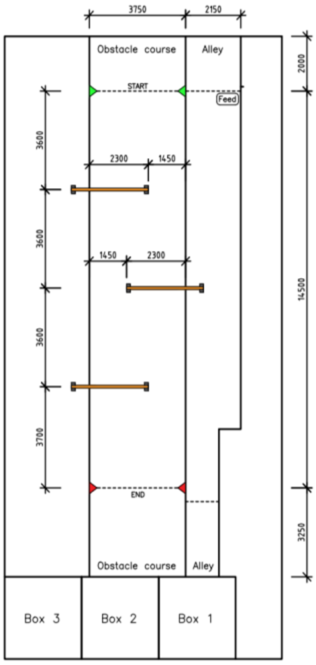

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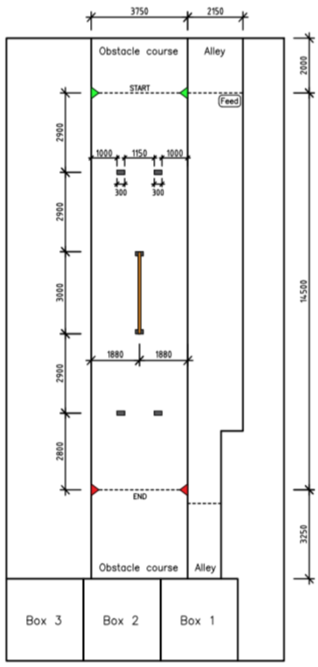

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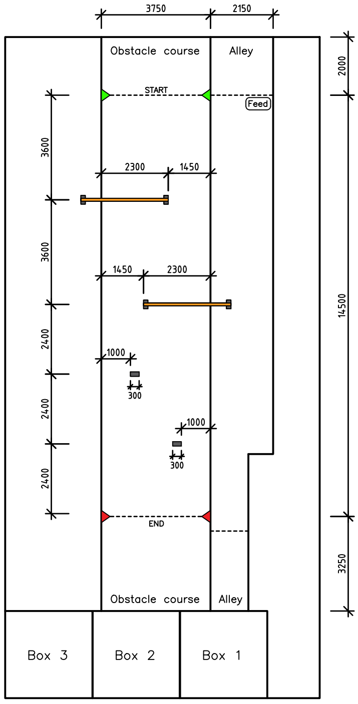

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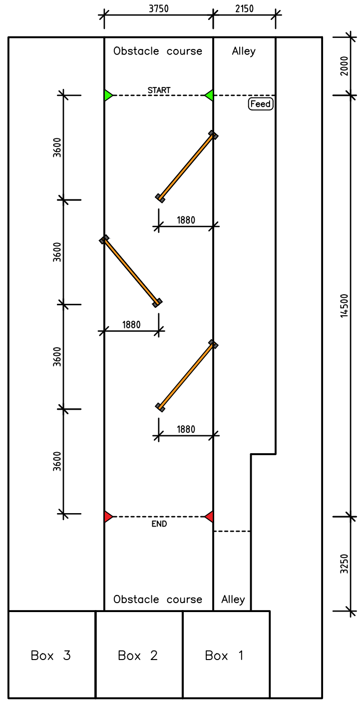

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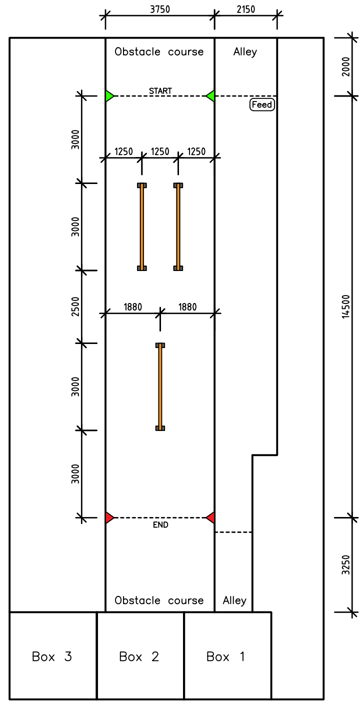

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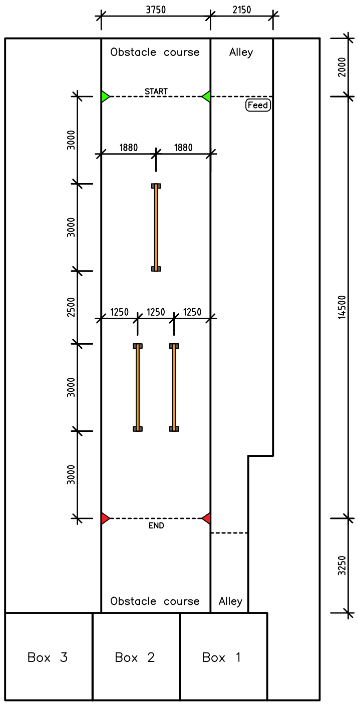

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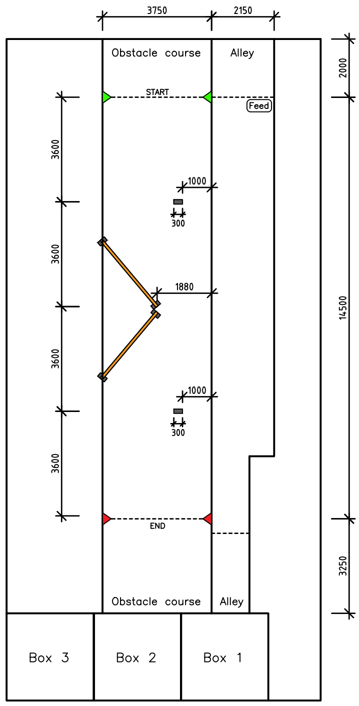

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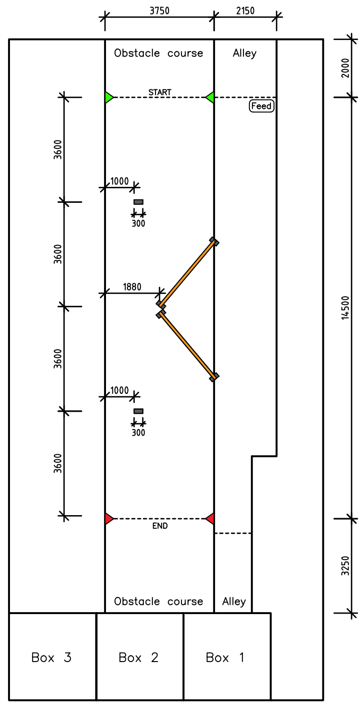

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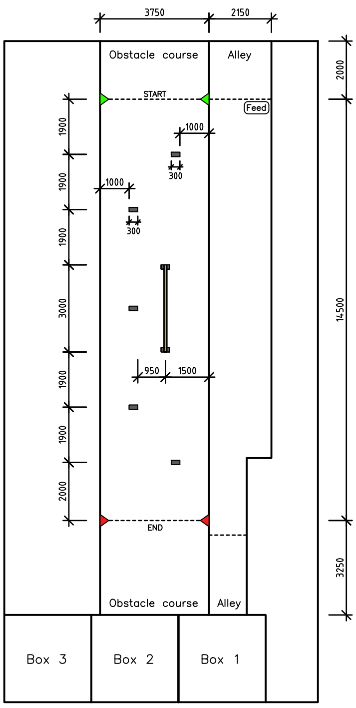

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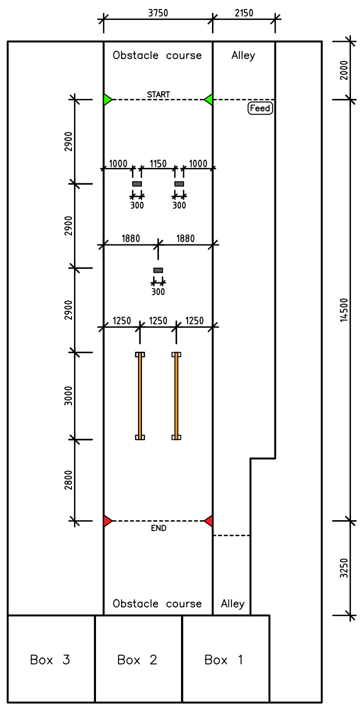

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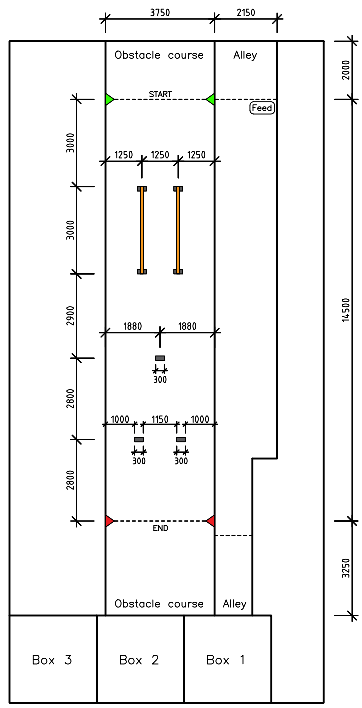

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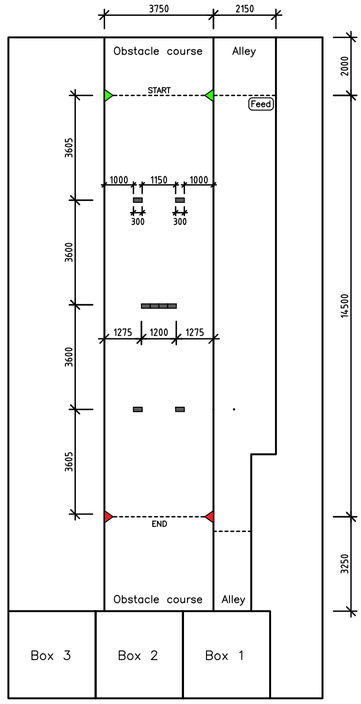

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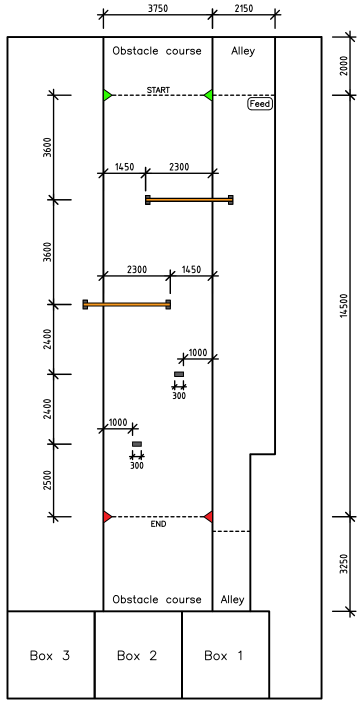

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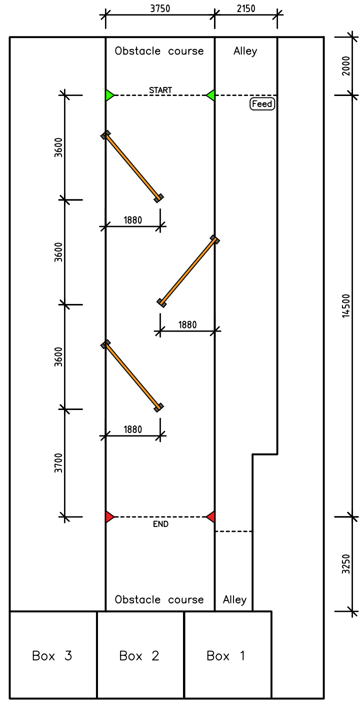

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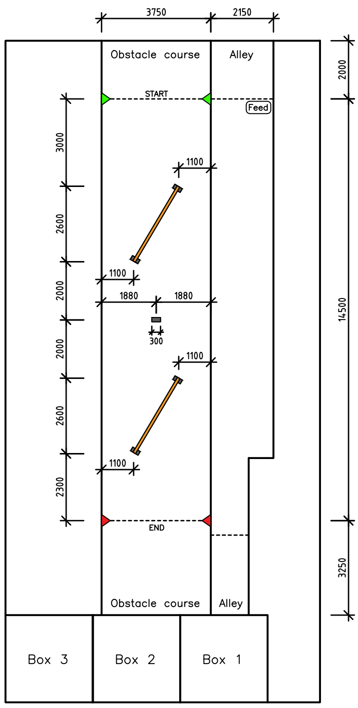

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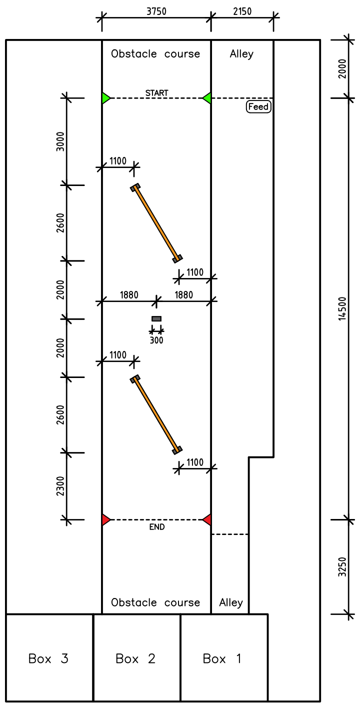

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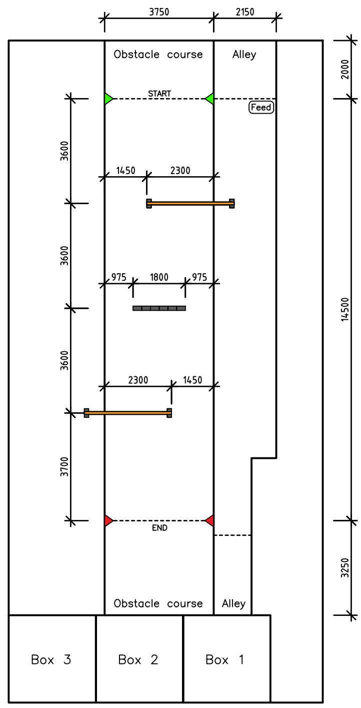

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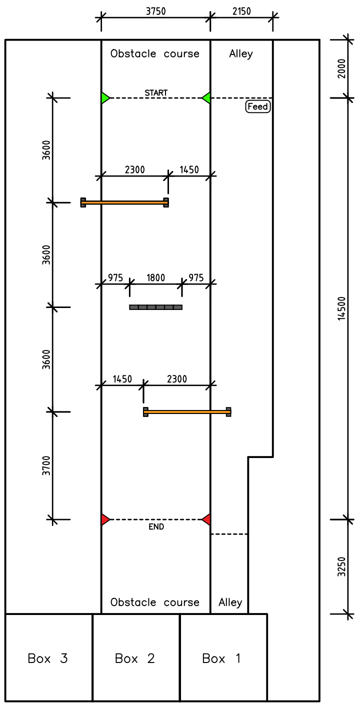

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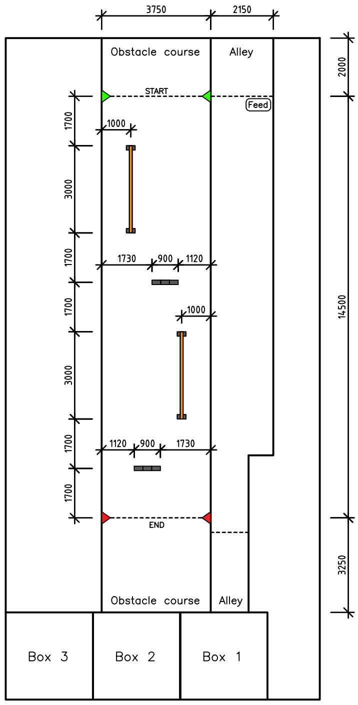

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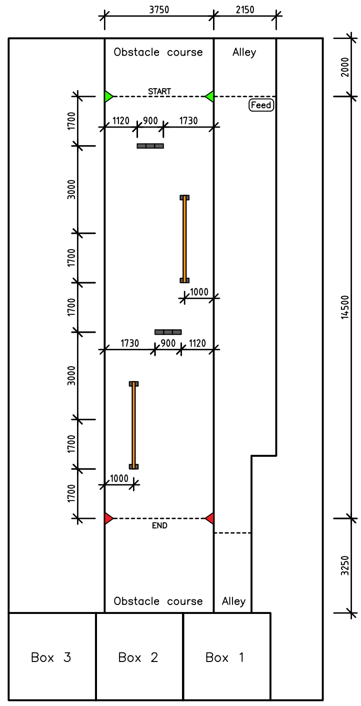

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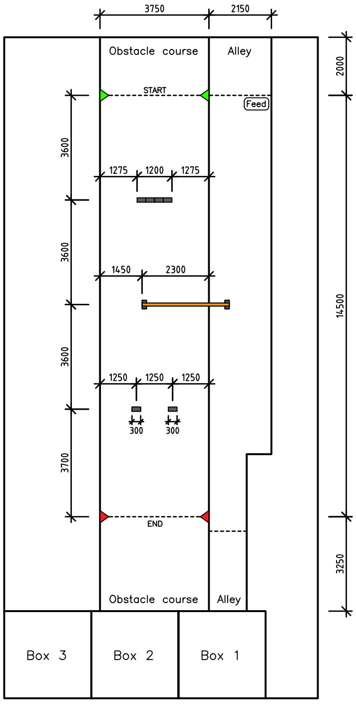
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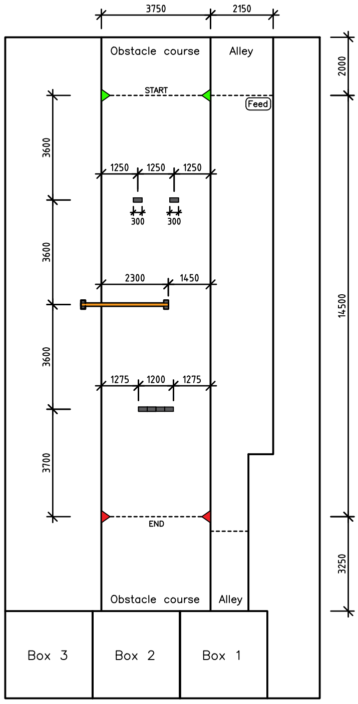
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300
3600
2300
1450
14500
3600
1275
1200
1275
3700
END
3250
Obstacle course
Alley
Box 3
Box 2
Box 1