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Faculty of Veterinary Medicine and Animal Science

Effects of Lighting on Heart Rates and Respiratory Rates in Calves

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Ljusintensiteters påverkan på hjärt- och andningsfrekvenser hos kalvar

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Abstract

This study aims to see if light intensities can affect calves' physiological reactions to their environment, in this case an obstacle course and a novel-object test. An additional red light setting compared to a white light was also tested in low light intensities. Twelve 2–4 month old heifer calves were used. Two tests were performed in succession; an obstacle course followed by a novel-object test, under four light intensities; 225±20 lx (full), 5±0.7 lx, 0.5±0.2 lx and 0.5±0.2 lx in red light. Heart rate and respiratory measurements were taken before and after the obstacle course and after the novel-object test. The overall results showed an increased HR in 225 lx and 0.5 lx in red compared to all other light intensities ($p<0.05$), whereas 5 lx showed the lowest heart rate compared to all other light intensities ($p<0.05$). Results from measurements taken before the obstacle course showed the largest increase in heart rate in 225 lx and 0.5 lx red ($p<0.05$). For measurements taken after the obstacle course, 225 lx gave the highest heart rate course ($p<0.05$), 0.5 lx and 0.5 lx in red were intermediate ($p<0.05$) and 5 lx gave the lowest ($p<0.05$). After the novel-object test there were no differences in hart rates, these were all lower than both before ($p<0.05$) and after ($p<0.05$) the OC. There was no effect of light intensity on respiratory rate. However, the respiratory rate was higher after the obstacle course independent of light-intensity (i.e., treatment) ($p<0.05$). There was no difference in respiratory rate for the measurements taken before the obstacle course and after the novel-object test. In conclusion, 225 lx and 0.5 lx red gave an increase in heart rate in calves before the obstacle course. After the obstacle course, the full lighting (225 lx) had an increasing effect on heart rate, while the effect of both low light intensities (0.5 lx and 0.5 lx red) heart rate was intermediately high. There were no treatment effects on heart rate during the novel-object test. There were no treatment effects on respiratory rate at any measuring point.

Sammanfattning

Syftet med den här studien var att se om olika ljusintensiteter kan påverka kalvars fysiologiska reaktion på sin omgivning, i det här fallet en hinderbana och ett novel-object-test. I låga ljusintensiteter testades även rött ljus som komplement till vitt ljus. Till studien användes tolv stycken 2-4 månader gamla kvigkalvar. För att testa kalvarnas fysiologiska respons i 225±20 lx, 5±0,7 lx, 0,5±0,2 lx och 0,5±0,2 lx med röda lampor, användes en hinderbana direkt följd av ett novel-object-test. Hjärt- och andningsfrekvenserna mättes före och efter hinderbanan samt efter novel-object-testet. Resultaten visade att 225 lx och 0,5 lx med röda lampor gav störst ökningar i hjärtfrekvens (HR) överlag ($p<0,05$), medan HR var lägst i 5 lx ($p<0,05$). Resultaten från mätningar innan hinderbanan visade att 225 lx och 0,5 lx i rött gav högst HR ($p<0,05$). Resultaten från mätningar efter hinderbanan visade att HR var högst i 225 lx ($p<0,05$), medan HR var mittemellan i 0,5 lx, rött, och 0,5 lx ($p<0,05$) och lägst i 5 lx ($p<0,05$). Det fanns inga signifikanta skillnader i HR mellan de testade ljusintensiteterna i mätningar tagna efter novel-object-testet. Ljusintensiteterna gav heller ingen signifikant effekt på andningsfrekvens. Den effekt som kunde ses på andningsfrekvensen var att den hade ökat efter hinderbanan jämfört med innan. Efter hinderbanan sjönk andningsfrekvensen. Slutsatsen är att 225 lx och 0,5 lx rött gav en ökad HR före hinderbanan. Efter hinderbanan ökade 225 lx HR hos kalvarna, medan HR var medelhög i de lägsta ljusintensiteterna (0,5 lx och 0,5 lx rött). Ingen av ljusintensiteterna hade någon effect på HR under novel-object-testet. Det fanns inga behandlingseffekter på andningsfrekvens vid någon av mätpunkterna.

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1. Introduction

The Swedish Animal Welfare Protection Act states that dairy cows should have dim lighting during the dark hours of the day (SJVF 2010:15). It does not state, however, why this is necessary nor which dimmer light intensities to use. The act makes no specifications concerning calves, but if there is reason to use dim lighting for cows, the same might apply for calves. Light intensity, or luminance, is measured in lux (lx) and is the measurement of how much visible light is present at the measuring point. Other countries, such as the UK, recommend that calves shall be provided with artificial lighting from at least 9:00 am to 5:00 pm (Welfare of Farmed Animals Regulation, 2000). The EU recommends that calves have a well-lit environment for at least 8 h per day (Recommendation Regarding Cattle, 1988).

Importantly, Swedish consumers often consider animal welfare buying animal products (Beck-Friis, 2015). For farmers, it is important to raise healthy calves that will become high-producing milk cows, and giving the calves a good start in life can help accomplish that goal. If night-time lighting is shown to stress calves, there is a possibility that having lights lit during the night could also affect calves negatively. If having dim lighting during the night is redundant, turning off the lights at night may help farmers save money.

Stress is a reaction to a perceived threat from which an animal wants to escape. Stress can be measured both behaviorally and physiologically. Stress is frequently studied by using novel-object and open-field tests, both of which serve to induce a measurable reaction in the animal. Chronic stress, however, can lead to a decreased immune response (Keeling & Jensen, 2009) and increased aggression, and both are unwanted by farmers.

Long-day photoperiods (16 h light; 8 h darkness) can have a positive effect on milk yield in dairy cows (Dahl et al., 2000). Another study found that heifers subjected to long-day photoperiods attained sexual maturity earlier and were taller and heavier at first parturition than heifers subjected to a short-day photoperiod (16 h darkness; 8 h light) (Rius and Dahl, 2006). Calves kept in low light levels of 2 and 20 lx over a 3-week period have shown less playing behavior and more resting and ruminating behavior than calves kept in higher light levels. Dannenmann et al. (1985) suggest that calves are less likely to play in low light, since they cannot perceive the stimuli that would otherwise encourage them to play, e.g., other calves, licking objects or playing. An extended photoperiod, but not necessarily full lighting at night, seems to have positive effects on onset of puberty and growth (Rius and Dahl, 2006). Despite some knowledge on how calves are affected by dim light over an extended period of time (Dannemann et al, 1985), and how an extended photoperiod affects growth (Rius and Dahl, 2006), there is little or no research on the effects on calves of having either dim lighting or no lighting during the night. There is also very little known about which light intensities calves prefer or if they find a light or dark environment to be more stressful.

Having red lights at night is sometimes recommended by companies (Lely, 2016), as it is a color that humans can see, but cattle cannot. Using red lights would allow farmers to enter the building without the cows being disturbed. However, Phillips and Lomas (2001) found that calves have a stronger reaction to novel stimuli in red light than in blue or green lights, and toreros commonly use red cloths in bull fights as the color is believed to aggravate the bulls (Riol et al., 1989).

This study aimed to see if light intensities can affect calves' physiological reactions to their environment, in this case an obstacle course (OC) and a novel-object test (NOT). An additional red-light setting compared to white light was also tested in low light intensities.

2. Literature review

2.1 Vision and light

In order for an animal to perceive light as images, light has to pass through the cornea and lens of the eye and reach the retina. In the retina, the different photoreceptors: rods, cones and photosensitive retinal ganglion cells (pRGC; Van Diepen et al., 2015), react to different wavelengths of light and send signals through the optic nerve to the visual association cortex in the brain (Sjaastad et al., 2010), where the signals are interpreted as images.

Rods function at low light levels and cones at intermediate to high levels of light. At intermediate light levels, rods and cones work together to give the animal vision. The light level or frequency at which rods or cones function depends on their morphology and biochemical properties (e.g., the amount of visual pigment inside the cones) (Peichl, 2005). The pRGCs are responsible for vision in high irradiance and are thought to relay information about light over a longer period of time than either cones or rods (Van Diepen et al., 2015). The eyes' ability to relay information about the amount of light is important in setting an animal's circadian rhythm. When no light hits the retina, signals are sent to the hypothalamus signifying that it is dark. The hypothalamus in turn signals to the pineal gland to release melatonin. In diurnal animals, such as the cow, this makes them drowsy, whereas nocturnal animals, such as the rat, become more active (Schomerus and Korf, 2006). Van Diepen et al. (2015) argue that signals from the rods, cones and pRGCs work together to set the circadian rhythm. Each photoreceptor type works best at different wavelengths of light, and the differences in daylight color composition can thus give animals a sense of time of day.

2.1.1 Color Vision

Most mammals have two cone classes that have either long-wave sensitive (LWS) opsins or short-wave sensitive (SWS1) opsins, resulting in dichromatic vision. They have the ability to discriminate long from short wavelengths, i.e., red and blue light, but not between the longer or shorter wavelengths, e.g., red and orange or green and blue. In primates, a mutation to the LWS opsin enables us to see green and red light separately (Peichl, 2005).

To find out more about exactly which colors cows can perceive, Dabrowska et al. (1981) used 11 cows of Lowland Black-and-White breed aged 3–5 years. Cows were rewarded with food for choosing correctly between seven colors from the Oswald's scale and 16 gray shades from the Hering's scale. The colors were yellow, pink, red, violet, blue, green and yellowish-green. During the test, a randomly chosen colored card and a grey card were each hung in separate openings. The cows had to stick their heads through the correct opening and touch the card for a feed award. If the animals picked a colored card less often or as often as a gray card, this would indicate that they could not perceive that color. All colors tested were deemed perceivable by the researchers. Out of the seven colored cards, the blue card was the hardest to distinguish from the gray ones. It was chosen correctly 63.3–97.1% of the time by the 11 cows. Yellowish-green was the easiest to distinguish from the grey cards and was chosen correctly 90-100% of the time.

Gilbert and Arave (1985) also showed that cows have the ability to discriminate between different colored lights by teaching Holstein heifers to press a plate with the correct color stimuli. The colors used were red (610 nm), green (538 nm) and blue (492 nm). The heifers were simultaneously presented with two colored lights. Two out of eight heifers were not able to distinguish any colors from another and didn't seem to understand the task at hand. The remaining six heifers were able to discriminate between all colors, but had most trouble choosing correctly between green and blue. The colors were tested in the

same order for each animal, thus the result of the last test may have been influenced by a change in which color was deemed "right" in previous tests.

Phillips and Lomas (2001) performed a similar study, where they assigned 11 calves to three groups. Each group was taught that either blue (415 nm), green (525 nm) or red (635 nm) wavelengths was the correct color, when compared to a white light of the same intensity. At the end of a 5-day testing period, the calves chose correctly between red and green 89% of the time, between red and blue 82% of the time and between blue and green 52% of the time. The calves were thus able to distinguish between red and green, and red and blue, but not blue and green. Further, the calves were tested in a novel-object test, where the same three colors of light were used as background light, with no additional white lights. The calves were more active and showed stronger movements in the red light compared to the blue and green lights.

Red lights are sometimes used in light experiments when technicians need to work with animals in darkness without interfering with light schemes (e.g., Lawson and Kennedy, 2001; Muthuramalingam et al., 2006). A similar practice is recommended by Lely (2016) when farmers inspect their cows at night, as cattle lack specific cones for red light and thus won't be disturbed by the light the farmer is able to see.

2.1.2 Effects of lighting

Some species of animals show clear effects from different light intensities. Rats are nocturnal, prefer dim lights and are easily disturbed by bright lights. Juvenile rats accustomed to dim lights will stop playing if lights are too bright (Castelhano-Carlos and Baumans, 2009). Hens and broilers are also easily affected by light, and it is common practice to use light regimes for these throughout production (Olanrewaju et al., 2006). The need for light regimes in poultry production is especially important, as a higher light intensity (30 lx vs 3 lx) is believed to give rise to more severe feather pecking (Kjaer and Vestergaard, 1999). The effect of light on cattle and calves seems less pronounced, and light regimes are not commonly used. The effect of photoperiods in cattle have, however, been studied quite extensively.

Ruis et al. (2005) found that heifer calves exposed to 16 h of light (450 lx) and 8 h of darkness (0 lx) grew faster than heifers with a reverse light scheme (8 h light, 16 h darkness) when fed a diet supplemented with rumen-undegradable protein (RUP). Heifers exposed to the long-day photoperiod also had an earlier onset of puberty than did short-day photoperiod heifers.

Muthuramalingam et al. (2006) found that 50 lx at night was the lowest level of light to inhibit melatonin levels in dairy cattle when testing 0, 5, 10 and 50 lx. During 16 h, in the day, 12 Holstein heifers were subjected to 200 lx. Having 50 lx at night suppressed the melatonin levels by around 48% after an hour compared to the other intensities and 45% the second hour. The suppressive effect only lasted for two hours during the treatment. The other three treatment levels had no effect.

Lawson and Kennedy (2001) similarly showed that light levels of ≥50 lx were enough to suppress melatonin levels during night in Holstein heifers. Of the 5 treatment levels tested; 0, 50, 100, 200 and 400 lx, only 400 lx was strong enough to sustain a suppressed melatonin inhibition throughout the night. However, the melatonin levels increased during the night for all treatments, with the greatest increase for 0 lx and the least for 400 lx. The researchers also measured blood hematocrits and found no difference in levels between groups, indicating that neither dark nor light was stressful to the cows.

Calves kept in low light levels of 2 and 20 lx over a 3-week period have shown less playing behavior and more resting and ruminating behavior than calves kept in higher light levels (100 and 130 lx). As previously noted Dannenmann et al. (1985), found that low light levels suppresses play behavior in calves.

Tests on adult cattle have focused more on the ability to walk from one place in the barn to the other. Cows that were made to walk through a passageway in different light intensities ranging from 0 to 250 lx

had the same walking rate but a higher stepping rate with shorter strides in the dark intensities. There was not a light intensity in which they refused to walk (Phillips et al., 2000)

2.2 Stress responses

A stressor is a perceived threat to the animal's well-being or homeostasis. As an animal perceives a threat, acute stress sets in. Heart rate (HR), blood pressure (BP) and respiratory rate (RR) elevate via the sympathetic nervous system, while gastrointestinal activity slows, due to decreasing innervations from the parasympathetic nervous system. At the same time, the hypothalamic-pituitary-adrenal axis (HPA-axis) is stimulated, and adrenocorticotropic hormone (ACTH) and glucocorticoids (e.g., cortisol) are secreted from the adrenal gland. These factors together heighten the animal's alertness and thereby the possibility to flee from the perceived threat. For the animal to be able to flee, the HR and RR increase in order to oxygenate the blood (Sjaastad et al., 2010).

If an animal experiences long-term threat, chronic stress develops, this can affect the brain's morphology and lead to increased aggression and anxiety (Wilcox et al., 2013). Chronic stress can also lead to stomach ulcers, cardiovascular disease and a defective immune system (Keeling & Jensen, 2009). Keeping chronic stress to a minimum by different means should therefore be a goal for farmers who want to keep their animals healthy. Correct handling of animals (Schmied et al., 2008), one that allows social interactions (Ekkel et al., 1995) and possibly the use of lighting schemes, could lead to healthier animals.

Stress responses can be measured in a number of ways, both behaviorally and physiologically. HR, RR and BP are common, noninvasive physiological factors that are measured in stress studies. HR can be measured manually with stethoscopes or externally with ECG monitors attached to the cows with a belt (Hopster and Blokhuis, 1994). Measuring stress hormone levels through blood or saliva samples can in itself induce stress if the animals are not used to handling. It is also possible to measure cortisol levels in fecal samples, but can be quite complicated, especially when animals are group housed.

2.2.1 Heart Rate

As mentioned, the sympathetic nervous system is the main activator of the heart. A stressor will increase the sympathetic innervations and increase HR, among other things. HR can thus be an indicator of stress, since an animal during rest will have higher innervations via the parasympathetic nervous system, which decreases HR (Sjaastad et al., 2010).

Emotional states affect physiological parameters, as could be seen in a study by Reefmann et al. (2009); in the study 14 sheep were trained to anticipate a control diet, which was dispensed after waiting for six minutes by the trough while the feed dispenser was turned on and made a humming sound. The sheep's physiological responses to anticipation and to receiving three different feeds were measured. The feeds were either the sheep's usual feed (control), unpalatable wood chippings to induce a negative emotional state, or an enriched feed for a positive emotional state. The physiological measures included HR and RR as well as body humidity, HR variability and body-surface temperature; these were measured via an electrocardiogram-recorder and a respiratory belt. The sheep had a significant increase in HR (+4–5 bpm) when they received the wooden chippings, compared to the anticipation phase. The heart rate decreased when they received control feed (-3 bpm) and increased slightly (+1 bpm) when receiving the high-quality diet.

In a study by Stewart et al. (2013), 40 Holstein-Friesian heifer calves aged 2 and 5 days were either positively (gently) or negatively (roughly) handled for a period of 5 weeks. At the end of the period the calves were exposed to two types of routine husbandry procedures on different days. On the first day, ear tags were attached. Two days later they received local anesthetics and were disbudded. During ear-tagging and disbudding, HR was measured using Polar© HR monitors. The electrodes were attached to a belt strapped around the calves' thorax. The fur underneath the belt was clipped prior to belt attachment to

enable contact between the electrodes and the skin. The average basal HR was 88.4 bpm for the positively handled group during ear-tagging, and 83.1 bpm in the negatively handled group. During disbudding, the positively handled group had a baseline of 87.7 bpm, while the negatively handled group's baseline was 87.2 bpm. HR was significantly higher during all treatments compared to the baseline, but no difference could be seen between the negatively and positively handled groups. Both ear-tagging and disbudding are thus seen as stressful regardless of previous handling.

In an experiment by Hopster et al. (1995) clear signs of acute, but short-lived, stress were observed in dairy cows when separated from their calves. When the calves were removed from the cows after 48–72 hours of being together, the cows' HR increased from an average of 81 bpm to 96 bpm. After the commotion of removing the calves, the HR decreased to 88 bpm after just two minutes of separation. There was no significant difference between HRs 5 minutes before and 5 minutes after separation. There were no significant increases in plasma cortisol levels, and the cows mainly called for their calves during the first 5 minutes after separation, before settling down. This experiment demonstrates that short-lived stress can be more easily seen in HR than with hormonal measurements (Hopster et al., 1995).

2.2.2 Respiratory rate

As with HR, RR is an indicator of stress, since the sympathetic nervous system activates the airways and lungs primarily to oxygenate skeletal muscles for rapid flight. An increase in RR can, however, be seen as an after-effect of an increased HR and physical activity, since an increase in arterial CO_2-concentrations is the main regulator of RR (Sjaastad et al., 2010).

During the aforementioned anticipation trial with the sheep, Reefmann et al. (2009) found that RR, like HR, increased significantly (+0.19 breaths/s) when receiving wood chips, compared to the anticipation phase, which showed that the sheep were disappointed in receiving unpalatable feed. RR decreased compared to the anticipation phase when the sheep received either the control (-0.81 breaths/s) or the high quality feed (-0.21 breaths/s).

Stewart et al. (2013) conducted a study looking at how different negative or positive handling might affect signs of stress, in this case RR, in calves while being ear-tagged and disbudded. During ear tagging the RR increased by 3.4 breaths/min for the positively handled groups, and by 4.8 breaths/min for the negatively handled group. Neither increase was statistically significant. During the disbudding the RR increased significantly from the basal value by 8.2 breaths/min (positive) and 9.3 breaths/min (negative). Although the team found no differences between handling groups, disbudding caused the RR to increase from the basal value, indicating that the treatment was stressful.

2.3 Novel-object tests

During novelty tests, the animals' behavioral and sometime physiological responses are recorded and evaluated (Van Reenen et al., 2005). Behaviors such as vocalizations, defecations, exploratory and avoidance can be recorded in order to evaluate the animals' stress responses when presented with a novel area or object (Philips & Lomas, 2001; Van Reenen et al. 2005). Usually, NOTs are performed in order to test if an event or a situation is stressful. The more stressed the animal is, the larger the reaction to the object ought to be.

There are a number of different ways to present objects to animals. The objects may already be in the test area before the animal enters (e.g., Leiner and Fendt, 2011), pop up as the animal is performing another behavior, e.g., when feeding (Dalmau et al., 2009), or descend from the ceiling after a few minutes in a pen (Van Reenen et al., 2005).

When administering an anxiety-reducing drug, brotizolam, to calves, Van Reenen et al. (2005) found that the drug casued a significant increase on the animals' locomotion when presented with a novel object and

willingness to contact an object compared to a control group. The animals administered the highest drug doses had significantly faster decreases in plasma cortisol after the NOT than did the controls. There was no difference in average HR between treatments, but differences could be seen when corrected for time spent in locomotion. When corrected for locomotion, the HR was significantly lower in calves treated with high doses of the anxiety-reducing drug. Anxiety, or fear, seems to inhibit the animals' locomotion and curiosity, whereas the drugs allow them to act on their curiosity (Van Reenen et al., 2005). Monitoring heart rates and behaviors can thus tell us about anxiety or stress in animals.

Leiner and Fendt (2011) saw clear fear responses in eighteen 2.5-year-old stallions in both behavior and HR when introducing them to two novel objects; an orange tarp and a white-and-blue umbrella, in a familiar paddock. During the first day of testing both the objects were novel to the horses which had been fitted with a HR monitoring belt. During the subsequent 5 days the horses were habituated to the umbrella, but not to the tarp. After the habituation process, on the last day of testing, the horses showed stronger reactions to the re-introduced tarp than the umbrella. They had a quicker increase in HR and showed more avoidance behaviors to the tarp than to the umbrella. This meant that even though they had accommodated themselves to the situation via the habituation to the umbrella, the tarp was still stressful to the horses.

3. Material and methods

3.1 Animals and housing

Twelve heifer calves aged 3±1 months from the cattle facility at Lövsta research centre, SLU Uppsala, Sweden, were included in the experiment. Eight of the calves were Swedish Red breed (SRB) and four were Swedish Holstein. All animal handling was approved according to the general ethics approval of education at Lövsta research centre. The calves were allocated to four groups of three calves. Two groups were tested per week. The testing barn had three pens; two calves were housed in each pen during the test weeks. The calves were given ad lib access to roughage and water, and concentrates were provided twice a day. The pens were cleaned twice daily. Registrations took place between 8:00 AM and 6:00 PM. The calves were left alone between 7:00 PM and 7:00 AM.

3.2 Treatments

Three white-light intensities were tested, including full lighting at 225±20 lx as a control, 5±0.7 lx and 0.5±0.2 lx. Red lighting was tested at one light intensity; 0.5±0.2 lx. The control lighting averaged 225 lx throughout the obstacle course (OC) and in the NOT pen.

All groups were tested once per light treatment, including the control, OC and NOT. Thus all groups were subjected to all treatments once. The order in which the light treatments were used was decided by a Latin square (4x4), see Appendix 1. Depending on the time it took to rearrange the lights between treatments, either one or up to three tests were performed in a day, meaning that a group of calves could either be tested 0, 1 or 2 times in a day. The calves were always allowed to rest between tests as the groups alternated throughout each week of tests.

The 5 lx intensity was obtained by covering the fluorescent ceiling lights with a layer of dark-plastic bags. To obtain an even distribution of light throughout the OC and in the NOT pen, holes were made in the plastic bags where needed and white LED light strips (Rusta AB, 7717-1365, Upplands-Väsby, Sweden) were lit. A lux meter (Clas Ohlson, Model 1300, Insjön, Sweden) was used to check the light intensity along the OC and in the NOT pen. To obtain the most even lighting conditions along the course, the light intensity was measured at several sites and extra light strips were used where necessary to increase the

light intensity. Wherever the measured lux level exceeded the intended level, dark tape and dark plastic bags were affixed to existing lighting to make them darker. In this way a mean value of 5 lx along the course was obtained.

For the white 0.5 lx test, LED light strips were set up along the sides of the obstacle course and the NOT pen. The white lights were 0.5 lx brighter than the red ones and required dimming using dark adhesive tape and dark plastic bags to achieve the same light level. The red 0.5 lx intensity was obtained by using LED red-light strips (Konstsmide, 4610-550, Gnosjö, Sweden) consisting of white lights with red caps. These were set up alongside the white-light strips and did not need any additional dimming.

All lux measurements were taken at calf eye-level, approximately 50 cm above ground. The facility is equipped with lights that cannot readily be turned off manually; e.g., emergency exit lights; these were covered up to avoid light leakage. Some light came through the door cracks; however, this leakage was very small and far enough away to not have a measureable influence on the light levels in either the course or in the NOT pen.

3.3 HR and RR measurements

HR and RR measurements were taken before the OC, after the OC and after the NOT for each calf and light intensity. The RR was measured by feeling the exhalations with two fingers in front of one of the nostrils. This proved easier than looking at the flank for breaths, since the calves would not stand still on their own. HR was measured with a stethoscope at heart level while holding the calf still.

HR and RR were measured in the NOT pen, which was located by the OC finish line. Both HR and RR were measured for 15 seconds and then converted to beats or exhalations per minute. After the first HR and RR measurements, the NOT pen was opened into an aisle that led to the start of the OC. One handler walked in front of the calves and one walked behind the calves to the start of the OC. At the start of the OC, a gate was opened to let the calf out. When both handlers and calf had walked through the gate, the calf was allowed to cross the start line.

3.4 Obstacle course

The OC was built from cavaletti bars (Safety-System, 3.0 m light-weight boom, Enköping, Sweden) and blocks (Safety-System, Cavalettiblock small, Enköping, Sweden) for equestrian use. These were rearranged for each light intensity and group so that no calf would do the same course twice. Already existing interior fencing, as well as temporary fences set up along the side were used prevent the calves from straying off course. During the OC one handler walked in front of the calf and encouraged it to walk by rattling a bucket of feed or by skipping in front of it, depending on the calf's preference. At the same time the second handler walked behind the calf in the OC prevent it from turning around and going back as well as to encourage it to continue walking without stopping for extended periods of time (>10 s). After the calf had passed the goal line it was led to the NOT pen where HR and RR were measured a second time (see Figure 1).

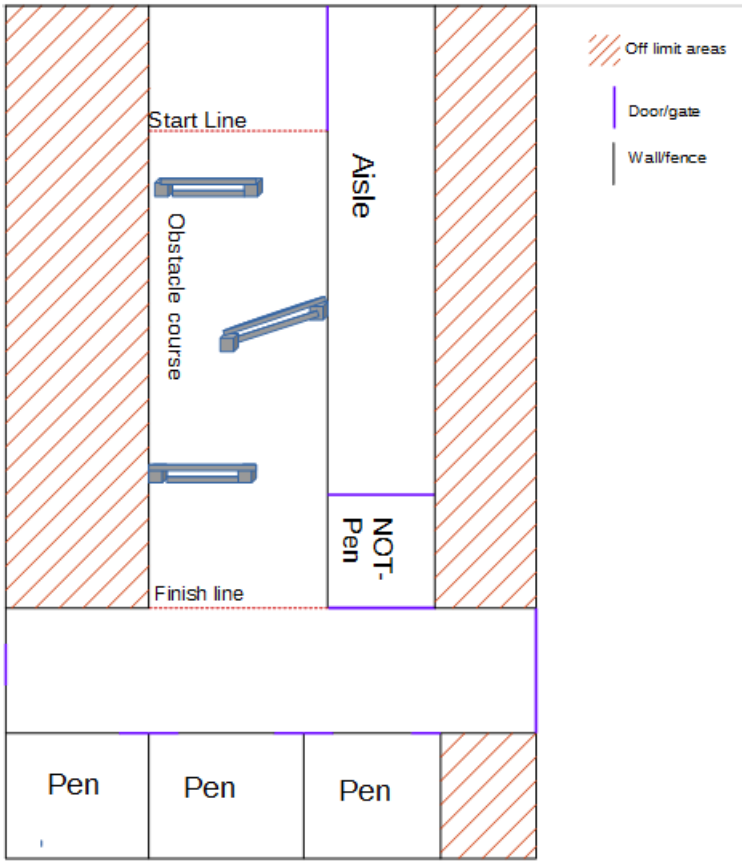

Figure 1. Drawing of the stable in which the experiment was conducted. The drawing is not to scale.

During the first week the calves were allowed one practice run through the aisle and obstacle course in full lighting. After the test run, and between groups, the OC's layout was changed to ensure that the calves did not memorize the course.

During the practice run all calves skipped enthusiastically through the course. However, it became apparent during the first recorded tests of groups 1 and 2 that the novelty of the course had worn off. This caused most of the calves to take longer than 8 minutes to complete the course and these runs had to be interrupted. The first tests of groups 1 and 2 were discarded and redone later during the week, after the calves were trained to follow the sound of a rattling bucket of concentrate feed. Once the calves understood to follow the handler with the bucket, the testing resumed. During the second week, a full day was needed before the tests began to train the calves in groups 3 and 4 to follow the handler as they shook a bucket of concentrate. The animals in groups 3 and 4 were younger than the ones in groups 1 and 2 and were not as accustomed to eating concentrates. They did however quickly understand the idea of following the handler with the bucket through the OC (the layout of which was later changed before the first test).

3.5 Novel-object test

The NOT was performed directly after the second HR and RR measurements. A novel object was placed within eyeshot of the calves, either beside or in front of them, but never closer than 1 m nor further than 2 m. Seven novel objects were used in the NOT. These included an inflatable frog, a beach ball, a hard plastic toy cat, a small windmill, a pink plastic container and an orange traffic cone (see Appendix). None of the objects were anything the calves would have seen before in their life on the farm. For each group a

different novel object was used per light intensity, avoiding using the same object in the same light intensity for any two groups. However, during the last group of tests during the last week, one calf accidentally was presented with an object that it had already seen in a previous test. During the NOT each calf was kept in the NOT pen with the novel object for 15 min. During this time the behavior of the calves were recorded for a project run parallel with this one. Afterwards, HR and RR were measured a third time, before the calf was let back in to its home pen.

3.6 Data handling

Data from the HR and RR were statistically analyzed in SAS 9.4 (Statistical Analysis System, Cary, USA). A mixed linear model using Restricted Maximum Likelihood (REML) estimation was used to look for differences in physiological outcomes by treatment condition.

For both data sets, the main effects tested were light intensity, i.e., the treatment, and the three measuring points; Start (S), Finish (F) and NOT. The interaction measuring points*treatment were tested for HR as both effects showed significance. Calf (ID) was set as a fixed factor for the analyses. The variables were breed, age, order of treatment, week of treatment and group. The data are presented as LS means and standard errors. The level of significance was p=0.05.

4. Results

4.1 Heart rate

Table 1. LS means and standard errors for heart rates (HR, beats/min) of calves (n=12) measured at the three measuring points; Start, Finish and NOT for all treatments. The overall LS means of each treatment and measuring point are also presented. "R" signifies red lighting in the specified lux level. Within measuring points and light intensities, different letters indicate significantly different values (P<0.05).

Treatment (lx)	**Measuring points (HR)**			**Treatment, mean**	**Significance**
	Start	Finish	NOT		
225.0	96.4±4.3	111.0±5.3	88.3±3.8	98.6±3.0	a
5.0	86.7±2.7	95.7±4.4	85.3±3.0	89.4±2.2	b
0.5	95.0±3.5	107.7±6.3	89.3±3.9	97.3±2.9	ab
0.5R	102.0±5.4	106.3±5.9	88.7±4.0	99.0±3.2	a
Measuring Point, mean	95.0±2.1	105.2±2.8	88.0±1.8	-	-
Significance	a	b	c	-	-

For all treatments between the different measuring points there were significant differences in mean HR at the start, finish and NOT. HR was significantly lower at start than at finish. HR was significantly higher at F than at NOT (P<0.05). HR at NOT was in turn lower than at S (P<0.05) (Table 1).

The HRs of the calves were significantly affected by both treatment (P<0.05) and measuring point (P<0.0001). For all measuring points mean HR was significantly higher in 225 lx than in 5 lx (P<0.05),

and significantly higher in 0.5 lx in red than in 5 lx (P<0.05) (Table 1). HR for 0.5 lx in white light did not differ significantly in any of the treatments.

There was no significant difference between in the mean measuring points (start, finish and NOT) (P=0.24), however, there were some significant interactions between *specific* treatments (light intensities) at the three measuring points. These interactions are shown in Figure 2.

For the measurements taken at the start, HR was significantly higher in 225 lx than in 5 lx (P<0.05). HR was significantly higher in 0.5 lx than in 5 lx (P<0.05). The HR was also higher in 0.5 lx red than in 5 lx (P<0.05) (Figure 2)

For the measurements taken at the finish, the only significant difference in HR was found between 225 lx and 5 lx (P<0.05). There was no significant effect of treatment on HR for measurements taken at NOT (Figure 2).

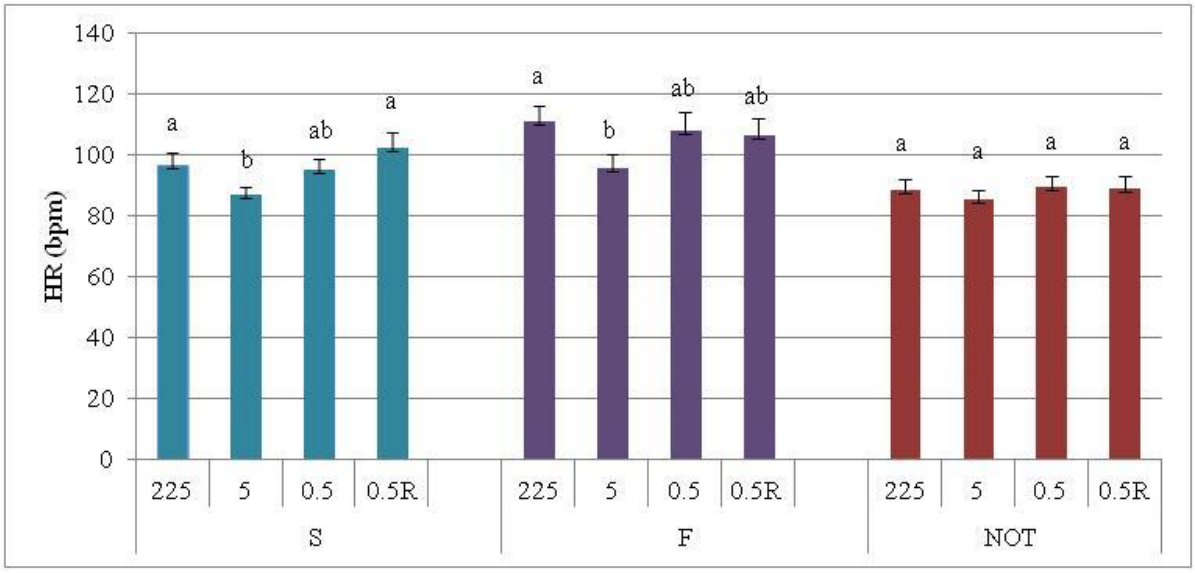

Figure 2. Interactions of treatments and measuring points. LS means for HR of calves (n=12) and for all treatments; 225 lx, 5 lx, 0.5 lx white and 0.5 lx in red light (0.5R) at the three measuring points; Start (S), Finish (F) and NOT. Values are presented as LS means, the error bars show the standard error. Within each measuring point different letters indicate significantly different values (P<0.05).

4.2 Respiratory rate

There was no significant difference in RR between treatments (P=0.7324). However, there was a significant difference between all measurement points; S, F and N (P<0.05). There was a significant difference between RR measurements taken at F and N (P<0.05) and between F and S (P<0.05), (table 2).

Table 2. Ls means and standard errors for respiratory rates (RR; in breaths/min) of calves (n=12) measured at the three measuring points; Start (S), Finish (F) and NOT for all treatments. The overall LS means of each treatment and measuring point are also presented. "R" signifies red lighting in the specified lux level. Within measuring points and light intensities, different letters indicate significantly different values (P<0.05).

Treatment (lx)	**Measuring points (RR)**			**Treatment, mean**	**Significance**
	Start	Finish	NOT		
225	27.3±2.3	27.3±2.4	27.7±3.1	27.4±1.5	a
5	25.7±1.4	29.3±3.1	27.7±2.6	27.8±1.4	a
0.5	29.2±3.3	32.0±3.4	29.3±2.4	30.2±1.7	a
0.5R	27.3±2.6	33.3±2.9	24.7±2.6	28.4±1.6	a
Measuring point, mean	27.4±1.2	30.5±1.5	27.3±1.3	-	-
Significance	b	a	b	-	-

5. Discussion

Both HR and RR increase in stressful situations as a fight-or-flight response (Lener and Fendt, 2011), when excited (Reefmann et al., 2009) and from exercise and increased activity (Sjaastad et al., 2010). It is therefore hard to draw any decisive conclusions from either HR or RR whether they indicate stress or not, as they may only be indicators of activity. Based on HR at the three measuring points in the OC and NOT, the calves did not have a significantly higher HR in the darkest light intensities (0.5 lx red) than in full lighting (225 lx), as these were in the same significance group. Interestingly, the HR in the individual light intensities was highest in 225 lx and 0.5 lx red before the OC. After the OC, HR was the highest in 225 lx, while HR for both 0.5 lx red and 0.5 lx was intermediately high. This could mean that the full lighting allowed the calves to see properly and move faster through the OC, raising their HR, while with the two dimmest lights (0.5 lx red and 0.5 lx) the course was hard to navigate and the HR therefore raised. Calves have been seen to show more playful behaviors in light intensities over 100 lx compared to 2 lx and 20 lx (Dannenmann et al., 1985), which might explain why the full light intensity led to a higher HR.

As 5 lx gave the lowest HR before and after the OC, it seems to have been a sufficient light intensity for the calves to see their surroundings and feel safe, but the low lighting may have inhibited their playfulness and thereby their speed through the course (Dannenmann et al., 1985). Since HR in 0.5 lx red was significantly higher than in 5 lx, and tended to be higher in 0.5 lx, it was likely an effect of low visibility and stress when moving through the OC. Also, as there were no significant differences in HR between light intensities after the NOT; this suggests that the elevated HR was a product of low visibility when navigating the OC, rather than low light intensities being a stressor. Calves mainly rest at night (Hänninen, 2007) and may not need to move from one place to another during the night. An extended photoperiod, but not necessarily full lighting during the night, seems to have positive effects on onset of puberty and on growth. Since light is needed for the pineal gland (hypothalamus) to track circadian rhythms, full, continuous lighting is not recommended (Dahl et al., 2000). It would be interesting to see in future studies if lower light intensities up to 5 lx during the night could disturb calves' sleep patterns.

There were no significant differences in HR or RR between 0.5 lx and 0.5 lx red at any measuring point. HR was not significantly different between 0.5 lx and 0.5 lx red after the obstacle course. Several studies

have suggested that cattle can differentiate between long (red) and short (blue, green) wavelengths within the visible light spectrum (Dabrowska et al., 1981; Gilbert and Arave, 1985; Phillips and Lomas, 2001), but these studies do not say how cattle perceive red light in their surroundings. The OC results seem to point to the increased HR as an indication of an increased stress level due to difficulties seeing the obstacles in low light intensities. Both Lawson and Kennedy (2001) and Muthuramalingam et al. (2006) used red lighting when taking blood samples during photoperiod studies when testing in 0 lx. The current study however does not indicate any difference in using red or white lights in low intensities when it comes to visibility.

The only significant change seen in RR was the increase after completing the OC (F) compared to both the measurements taken before the OC (S) and after the NOT. The raised RR after the OC (F) was an effect of exercise from completing the course (Sjaastad et al., 2010). There was no significant effect of treatment on RR. In the experiment conducted by Reefman et al. (2009), providing wood chips to goats instead of feed was enough to raise the RR by 0.19 breaths per minute. The lack of a treatment effect on RR after the OC could be due to inadequacy when feeling for exhalations with one's fingers compared to the respiratory belt used in the study by Reefman et al. (2009). That the calves would lick the hand taking the measurement complicated the procedure.

For both HR and RR measurements, it might have been useful to use a measuring belt for continuous measurements during the NOT, similar to the ones used on the stallions which were subjected to two novel objects (Leiner and Fendt, 2011) and the on sheep in the anticipation study (Reefman et al., 2009). It is possible that a potential effect on HR and RR due to the different light treatments when the calves interacted with the novel objects might have been observable with continuous measurements from a belt throughout the NOT. Instead, measurements were taken only after the NOT, by which time some of the animals had laid down to rest (on three occasions). Behavioral reactions to novel objects during the NOT have been covered by Battersby (unpublished). That both RR and HR were low at the end of the NOT, might indicate that the time allocated for the NOT (15 min) was too long to record possible significant HR and RR responses to the novel objects in the different light intensities. The stallions in the study by Leiner and Fendt (2011) had a rapid increase in HR at first sight of the novel object, which showed a trend of decreasing slowly over a three-minute period The HR in the stallions had larger increases at first exposure to a novel objectand decreased with the number of exposures. Rapid increases and decreases in HR could also be seen in the experiment by Hopster et al. (1995), where they separated cows from their calves. The cows' HR increased immediately at separation and had settled back to baseline levels after just 5 minutes of separation. The current study would have benefited from a shorter NOT time of a few minutes to be able to better record the responses at first contact with the methods used. The calves had settled down by the end of the NOT at which time the HR and RR measurements were taken. But if the light intensity on its own had been stressful, the HR ought to have remained elevated for the duration of the NOT or for as long as they were in the dark.

The HR was generally higher when measurements were taken before the OC than after the NOT. The act of moving the calves from the housing pen to the NOT pen (approximately 1.5–5 m depending on housing pen) before the test may have aroused the calves enough to raise the HR for the initial HR and RR measurements. The HR may also have been raised from the anticipation of having to complete the OC, similarly to how the anticipation of receiving feed increased the HR in sheep (Reefman et al., 2009). Whether calves anticipate being released in an OC as much as sheep anticipate the arrival of feed is not known. Potentially, HR and RR could have been taken in the housing pen prior to the OC, but as calves were housed in pairs it would have been inconvenient to the handlers to have to deal with a curious calf as well as the calf whose measurements were being taken. Thus the calves were moved to the NOT pen for the HR and RR measurement before the OC.

One major problem of the current study was trying to get the calves to move through the OC without the handlers being too pushy or themselves being too excited in their behaviors. It was occasionally difficult

to know whether the calf had stopped at an obstacle out of curiosity or fear of being surprised in the dark. Training the calves to walk to the end of the OC by enticing them with treats may have lowered this uncertainty by learning to trust their handlers. Conversely, teaching them to anticipate a treat for completing the OC may have raised the HR, leading to more uncertain results.

5. 1 Conclusion

In conclusion, 225 lx and 0.5 lx red gave an increase in HR in calves before the OC. After the OC, the full lighting (225 lx) had an increasing effect on HR, while HRs were intermediately high in both of the low light intensities (0.5 lx and 0.5 lx red). Red lighting does not appear to be more stressful than white lighting at the same intensity. There were no effects of treatment on HR during the NOT. There was no effect of treatment on RR at any measuring point.

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7. Appendices

Appendix 1. Latin square depicting the order of treatment for each group

	Week 1		Week 2	
Calf group	**A**	**B**	**C**	**D**
Light intensity (lx)	225	0.5	225	0.5
	0.5	225	0.5 R	225
	5	5	5	0.5 R
	0.5 R	0.5 R	0.5	5

Appendix 2. Novel objects

The objects used from right to left: plastic container; beach ball; inflatable frog; windmill; hard plastic toy cat; traffic cone.