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Swedish University of Agricultural Sciences
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Photo: Sabine Ferneborg

Pernilla Olsson

Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **470**

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Effekter av låga ljusintensiteter och rött ljus på hjärtfrekvens, blodtryck, andningsfrekvens och aktivitet hos mjölkkor

Pernilla Olsson

Handledare:
Supervisor: Sabine Ferneborg, SLU, Department of Animal Nutrition and Management
Bitr. Handledare:
Ass. Supervisor: Emma Ternman, SLU, Department of Animal Nutrition and Management
Examinator:
Examiner: Kjell Holtenius, SLU, Department of Animal Nutrition and Management
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Abstract

Today dairy farmers in Sweden are obligated to have lights turned on during night time in addition to the mandatory daylight inlet. This presents a cost for the production but many argue that it can be economically beneficial, especially in loose housing of dairy cows. In loose housing and even more so in herds with automatic milking, well functioning cow traffic during all hours of the day is crucial to optimise production. In order to keep the cow traffic flowing, lights may be an important management tool. It is also perceived among farmers with tied up stall that night light decreases the incidents of cows accidental stepping on their own or neighbours udders. In both these cases economical gain may be potential. On the other hand today it is not clear how well cows see in darkness or at which light intensity they start to distinguish their surroundings, thus it is not clear if night light is really necessary. It may well disturb the cows' hormone cycle, explicitly the melatonin cycle, or in other ways create stress.

The aim of this study was to investigate if stress related properties as heart rate, blood pressure and respiratory rate were affected in cows at different light intensities. Furthermore whether or not these parameters were affected by the presence of red light was investigated. The activity of the cows in different light intensities was also recorded with the help of activity meters attached to their right hind leg. The light intensities aimed for were 0, 5, 20 and 50 lux and the experimental design used was a Latin Square. In the study a total of twelve dry dairy cows were used and during the course of the study two tests were carried out; an obstacle course and a novel object test.

The results indicate that heart rate and blood pressure were not affected by different light intensities or the presence of red light, still respiratory rate was slightly affected by the presence of red light. Also the results show that 0 lux with red light increased heart rate, blood pressure and respiratory rate compared to 0 lux without additional light. This suggests that the cows can detect red light and may be stressed by it. However no differences were seen due to the different light intensities and therefore cows are probably not stressed by low light intensities. This make sense since different light intensities is a normal part of their life during the day. The activity meters showed that the cows tended to be lying down a greater proportion of the time in 50 lux, especially in comparison with 20 lux. This could be due to better visual acuity in 50 lux thus making the cows feel more secure.

Sammanfattning

Idag måste mjölkbönder i Sverige enligt lag ha nattbelysning i stallarna utöver det dagsljusinsläpp som är förpliktigat enligt lag. Detta utgör en kostnad för produktionen men många anser att det finns ekonomisk vinning att hämta, speciellt i stallar med lösdrift. I lösdrifter och framförallt i besättningar med automatisk mjölkning, är en väl fungerande kotrafik avgörande för att kunna maximera produktionen. I syfte att få ett bra flöde i kotrafiken kan ljus vara ett viktigt verktyg. I uppstallade besättningar upplevs också att ljus på natten förebygger att kor trampar på sitt eller grannars juver. I båda fallen finns potential till ekonomisk vinning. Å andra sidan är det idag oklart hur väl kor ser i mörker och i vilken ljusintensitet de börjar urskilja sin omgivning, därför är det inte säkert huruvida ljus på natten är nödvändigt. Det kan mycket väl vara så att kornas hormoncykel störs, särskilt melatonincykeln som är beroende av ljus, eller att ljus på natten på andra sätt skapar en stress hos korna.

Syftet med studien var att undersöka ifall stressrelaterade egenskaper såsom hjärtfrekvens, blodtryck och andningsfrekvens påverkas av olika ljusintensiteter. Dessutom undersöktes om dessa parametrar påverkades av närvaron av rött ljus. Kornas aktivitet i olika ljusintensiteter mättes också med hjälp av aktivitetsmätare som fästes på kornas höga bakben. De ljusintensiteter som eftersträvades var 0, 5, 20 och 50 lux och försöket designades utefter en så kallad romersk kvadrat. Under studien användes totalt tolv kor och två olika test utfördes; en hinderbana samt ett s.k. ”novel object test”.

Resultaten indikerar att hjärtfrekvens och blodtryck inte påverkades av varken ljusintensitet eller förekomsten av rött ljus, däremot påverkade förekomsten av rött ljus andningsfrekvensen något. Dessutom så visar resultaten att 0 lux med rött ljus ökar hjärtfrekvensen, blodtrycket och andningsfrekvensen i jämförelse med 0 lux utan något extra ljus. Detta visar att kor kan uppfatta rött ljus och att detta skulle kunna vara stressande för dem. Emellertid inga skillnader sågs pågrund av olika ljusintensiteter vilket tyder på att korna inte blir stressade av låga ljusintensiteter. Detta är rimligt då låga ljusintensiteter är en naturlig del av dagens gång. Vidare visar aktivitetsmätarna att korna tenderar att ligga ned en större del av tid vid 50 lux, speciellt i jämförelse med 20 lux. Detta kan bero på att korna har bättre synskärpa i 50 lux och därför känner sig säkrare. Eftersom att liggandet är ett prioriterat beteende för kor så kan 50 lux rekommenderas i stallarna under natten..

Table of content

1. Introduction

Sweden is a country proud of its good legal protection of animal rights in the form of a detailed Act of Animal Welfare (SJVFS 2010:15). Also there is a public interest and awareness in questions relating to these issues and there are many both voluntary organisations and different organisations in the line of business overseeing the development of the animal rights protection. Despite the best intentions from both decision makers and the public some laws have led to unpredictable consequences. One example can be seen as a result of the law that demands that all stables meant for livestock has to have day light inlet, which has caused welfare problems in the poultry industry. Uneven light inlet and high light intensities have been seen to cause stress in poultry with animal welfare problems such as feather pecking and in severe cases cannibalism as a result (Kjaer and Vestergaard 1999). Therefore it comes as no surprise that there is a big interest in evaluating and controlling the use and function of laws concerning these areas.

As briefly mentioned before, all stables or barns housing farm animals have to have day light inlet. In the case of dairy cows the law also states that dim light has to be provided during the dark hours of the day. Furthermore, the fixed lighting in the stable should not subject the animals to any discomfort (SJVFS 2010:15). However there are no limits regarding what light intensity that should be used.

The first automatic milking system in Sweden was installed 1998 and since then an ever growing proportion of Sweden's dairy cows are milked with the help of these machines (Pettersson and Wiktorsson, 1984). Partly as a consequence to this and partly due to other reasons, more and more cows are kept in loose housing systems, where well-functioning cow traffic is essential to the profitability of the farm. Cow traffic describes the way that the cows should move through the stable in order to get access to fodder and cubicles, and be milked along the way (Munksgaard et al. 2011). All cows have to be able to lie down at the same time, but there only needs to be one water cup for every tenth cow. Neither does it have to be room for all cows to eat at the same time if feed is offered *ad lib* (SJVF 2010:15). This in combination with trying to achieve an optimal use of the automatic milking system, that is 24 hour production, often leads to farmers wanting to have almost full lighting even through the night. Still energy is not cheap and keeping the lights on all night will have a great impact on the profitability of the farm if this does not lead to optimal cow traffic.

It is not possible to know exactly how cows see the world, but it is important that we understand how much they rely on their vision in the daily life. The understanding of cows' vision could help us to provide a better environment for the animals and also could prove to be a helpful management tool. All mammals have eyes that contain both rods, that are light sensitive and make it possible to see in darkness, and cones which make it possible to see shapes and colours in daylight (Dannenmann et al. 1985). Cows' eyes are big and have a high amount of rods, which should make it easier for them to see in lower light intensities (Phillips et al. 2000). Cattles' eyes also have a biological reflector system named *tapetum lucidum.* This system increases the visual sensitivity in dim light by reflecting the light a second time, stimulating the photo-reception once again. However in order to develop this system, some visual acuity have been lost (Sjaastad et al. 2003). It is the cones that provide the colour vision (Dannenmann et al. 1985) and while all primates are tricomatic and possess three kinds of cones, the cattle eye only possesses two kinds of cones, and is dichromatic. Therefore it is believed that cows cannot distinguish between green and red. In this case cones would rather give a more detailed vision in daylight, than colour vision (Sjaastad et al. 2003). Today we do not know for sure how well cows see in the dark and neither have we any consensus about cattle's colour vision. Still red light is sold to farmers and marketed as a sort of "invisible" light for cattle. If this is true red light could prove to be a useful management tool. Framers could for example inspect their cattle without disturbing them during night time.

The aim of this study was to investigate if stress-related properties as heart rate, blood pressure and respiratory rate, as well as the activity are affected in cows at low light intensities. Furthermore whether cows can detect red light or not was tested. The hope is that further studies of the vision in dairy cows can establish how well dairy cows can see in the dark and if night light at all is necessary both in an economical and biological point of view.

2. Literature review

2.1 Light intensities

Light intensity is measured with a light meter and in the metric system the unit is lux. Footcandles (fc) is also a unit used, describing the lumen per square foot while lux describes the lumen per square meter. One fc is 10.76 lux. Lumen measures the emitted visual light from a light source (Janni 2000). 0 lux is complete darkness with no inlet of day light or supplementary light.

In order to avoid a decline in number of visits to the automatic milking system (AMS) during the dark hours of the day, several Swedish farmers started to have full lighting both day and night when AMS was introduced. Pettersson and Wiktorsson (1984) tested whether dairy cows preferred dark (5-7 lux) or illuminated (app. 200 lux) resting areas during night time. In order to do so they used 46 cows in AMS with controlled cow traffic. The results did not show any differences between the use of the dark and illuminated side of the barn on herd level, but some individuals did prefer one side. Interestingly, some individuals preferred the dark side while others preferred the illuminated side. It was concluded that in 5-7 lux, the overall milking and feeding frequency or pattern was not affected.

Even though Pettersson and Wiktorsson (1984) did not see any conclusive differences, Phillips et al. (2000) saw that cows' step length decreased in low light intensity while the step rate increased. This was interpreted as the safest way for the cows to travel at the same speed but with less risk of slipping or encounter unseen obstacles. It was also suggested by the authors that step rate increased because the cows wanted to rejoin the herd faster. As the speed and stepping rate was lowest in 32 lux and the arc of travel of fore and hind limbs was at optimum in 119 lux, the cows seemed to be most comfortable walking in light intensities between 32 and 119 lux. Additionally, Phillips et al. (2000) postulates that cows may find darkness aversive.

In Norway, Reksen at al. (1999) concluded that dim light at night (4-160 lux, mean 36 lux, measured in feed alley) preferably but not necessarily together with a 12 h long photoperiod was related to higher milk production and better reproductive performance in winter time compared to farms that did not use light during the night. The authors hypothesized that using night light as well as a 12 h photoperiod imitate natural summer light pattern and therefore stimulates reproductive traits. Cows in herds that used lights (approximately 36 lux) in the barn during the dark hours of the day had fewer days open, shorter calving intervals, needed fewer AI per cow, also fewer cows returned into heat after insemination in comparison with herds that did not use lights during the night. Important to notice is that the improved reproductive performance was seen to a greater extent in heifers than in cows. Age at first AI and age at first calving was lower in farms using light during the night. After correcting for additional feed and energy cost, Reksen et al. (1999) calculated that a Norwegian farmer could gain $736/year by using light during the dark hours of the day together with 12 h long photoperiod.

Melatonin is stimulated by the absence of light and induces relaxation and sleep in mammals. For that reason there are some concerns that the presence of light during night could deprive the cows of rest (Lawson and Kennedy 2001; Muthuramalingam et al. 2006). In a study conducted by Lawson and Kennedy (2001) the results showed that melatonin levels dropped in heifers when 50 lux or higher was used during night. But after two hours of 50 lux the inhibition of melatonin seemed to disappear and only higher intensities could suppress the melatonin levels for a longer time and only intensities as high as 400 lux suppressed the melatonin level throughout the whole night (8 h). Similar results were seen by Muthuramalingam et al. (2006), who suggested that 10 lux or lower intensities could be appropriate to use in areas recommended to be dark, since no effect on melatonin levels were seen when using 10 or 5 lux in dairy heifers compared to 50 lux at night which suppressed melatonin levels at least the first two hours of exposure. Both studies showed a decline in melatonin concentrations between 50 to 70% when using intensities above 50 lux. Yet these results could not be repeated by Bal et al. (2008), who saw no difference in plasma concentrations of melatonin, IGF-1 or milk yield when comparing light intensities 0-5 lux with 40-60 lux during the night. Instead the results showed only a small increase in prolactin levels for 40-60 lux compared to 0-5 lux.

2.2 Colour vision

As mentioned, the eye contains both rods and cones and it is the cones that provide the colour vision (Dannenmann et al. 1985). Still it is not clear which colours that are visible to cows. Still it has been shown that vision dominates over hearing in feeding situations. Fifteen Holstein calves (app. 6 months old) were trained to touch a bar in response to either auditory or visual stimuli, in order to receive grains as reward. The auditory stimulus was a pure tone at 1 kHz and 75 dB sound pressure level (spl) while the visual stimuli were 10 W white, green or red light. The results demonstrate that the calves showed the correct response nine times more often in white light than due to the sound. The same figures for green and red light were 6.8 and 3.7 respectively. The authors suggest that this result also indicates a hierarchy between colours, white being easiest to recognize and red the hardest (Uetake and Kudo. 1994).

It has also been seen that cows can use colour of the clothes to distinguish between handlers in the barn and another study shows that calves behave differently in different coloured light; hence colour can have a great impact on cattle and their perception of their environment (Munksgaard et al. 1997; Phillips and Lomas 2001). Also when faced with a coloured card and a grey card of the same brightness as the coloured card, the cattle more easily distinguished red from grey and had trouble to discriminate blue from grey. Unfortunately the design of that experiment made it impossible to see if the cows could distinguish between colours and not only choose them over different shades of grey (Dabrowska et al. 1981).

A similar study conducted on eight cows gave comparable results. The cows had to choose between two feeding troughs marked with different colours. The troughs both contained feed, but one was made unavailable by a see-through plastic sheet. First discrimination between white and black was tested, obviously differing in brightness. For half of the cows the trough marked with white had available feed in it and vice versa. To conclude that the cows could differ between the colours, they had to choose the correct feeding trough (through with available feed) at least 75 % of the times. The percentage easily passed this threshold for distinction between white and black. Next step in the study was to have one trough marked with colour (red, orange, yellow, green, yellowish green, blue or violet) and the other was marked with a grey colour that had the same brightness as the colour compared with. The results showed that orange, red, yellowish green and yellow gave a test percentage exciding 75%, thus they were considered to be perceived by the cows. Violet, blue and green did not reach 75% and therefore whether or not the cows can distinguish these colours are not clear (Riol et al. 1989).

To see if cows could use colours as a visual aid to separate between different handlers one handler was wearing red clothes and the other handler wore yellow clothes. One handler was always gentle towards the cow and the other showed more aversive behaviour. Which handler that had what coloured clothes was randomized between the groups of cows. Still the cows always avoided the aversive handler. But since the cows did not avoid other people (keepers not working in the study) wearing the same coloured clothes as the aversive handler the recognition was not solely based on colour. However the study did not show that cows could distinguish between handlers wearing the same colour overalls either (Munksgaard et al. 1997).

In the study by Phillips and Lomas (2001) calves were trained to distinguish between long (635 nm, red), medium (525 nm, green) and short (415 nm, blue) wavelengths. The calves had to choose to enter one of two chambers with different wavelengths. In the chamber with the correct wavelength there was a bucket of concentrate available for the calf. In the incorrect chamber a bucket of concentrate was also presence but made unavailable to the calves by a net. This ensured that scent would not guide the calf to one of the rooms. Results showed that calves could distinguish between red and green light, and between red and blue light. But in most cases they could not perceive the difference between green and blue light. Phillips and Lomas (2001) calculated that the top limit for the calves vision were 620 nm.

The authors also observed behavioural differences in the different wavelengths. Three different tests were conducted; novel stimuli test where a rectangle was painted on the floor, fear test where a wooden board was dropped to the floor behind the calf and a response to handler test were the time it took for the calf to navigate between two barriers and reach the handler was measured. The results showed that during the novel stimuli test more activity was seen in red light. The calves also approached the handler faster in red light. In the fear test the calves were faster in green light than in blue light. This could be a sign of greater acuity in green light (Phillips and Lomas 2001).

2.3 Stress responses

Different physiological factors can be used in order to detect stress. Heart rate (HR), blood pressure and respiratory rate (RR) are examples of factors that are often used. They are relatively easy to measure and do not have to be invasive. Stress response is often investigated by collecting blood to analyse for different stress hormones, this require invasive methods that often persists longer than the stimuli and can in itself elicit stress response.

2.3.1 Heart rate

Increased heart rate (HR) is often translated as short-term stress. During human stroking of the cow decreased HR was interpreted as a feeling of contempt and lowering of stress, while a raise in HR due to novelty of the test was assumed to be a sign of anxiety (Schmeid et al. 2008). It has been seen in at least one study how the average HR increases from 60 to 86 beats/min (bpm) when cows were introduced to a new environment. A rise in HR (60 to 160 bpm) was also seen during agonistic encounters between cows. When the agonistic encounter was over, the less experienced cow went to lie down, which slowed the HR down , however the baseline level was not achieved until the more experienced cow (agonist) laid down 15 min later. It was concluded that these results support the notion that HR can be used to detect anxiety in cattle (Lefcourt et al. 1999).

If body position affects HR is not sure. There are results that show an increase in HR with 4.0 ± 1.4 bpm when cows stood up and a decrease with 4.8 ± 1.0 bpm when lying down (Lefcourt et al. 1999). Nevertheless no significant difference was seen in HR during a second study where cattle was claw trimmed, thus in lateral recumbency. The study evaluated stress in cattle during claw trimming and HR was measured to be 73 ± 3.5 bpm in the control group before the claw trimming started. The control was given saline solution while the other two groups were injected with a low dose (0.05 mg/kg bw) of xylazine (drug used for sedation) 15 min before lateral recumbency. Then the stress response was compared between the saline injected group and one of the groups injected with xylazine, while in lateral recumbency. The other group of cows given xylazine were kept standing as a control to the group in lateral recumbency. Rizk et al. (2012) hypothesis was that the cows injected with xylazine would show fewer signs of stress than the cows injected with saline solution. Indeed the result showed that cows injected with xylazine had lower hormonal and metabolic stress response (for example lower HR) than the cows injected with saline solution.

When using external monitors such as EKG or arterial pulse, the thickness of the hide and movement of the cattle can make it difficult to get good readings (Lefcourt et al. 1999). In the study mentioned earlier about the effect of human stroking on cows, HR was measured using electrodes attached to the cow with a girth. Two test sessions was conducted during the study. The first session was conducted without prior habituation to stroking, whereas the second session was conducted three weeks later. During the three weeks between the test sessions the cows had been stroked 5 min/day. The baseline for the different sessions differed somewhat; 67.3 ± 0.9 bpm in session number one and 72.6 ± 0.9 bpm in session number two, but it is not stated in the article whether this difference is significant or not (Schmeid et al. 2008).

To evaluate stress during milking HR was measured and used as an indicator. According to the results HR increased with 10 bpm when entering the milking stable; still this was within the normal variation (83.2 ±12.6 bpm) (Hopster et al. 2002). In another study by the same author the HR for cows with calf was recorded to around 81 bmp and then a sudden rise to 96 bpm was seen the first minutes after calf removal. Thereafter the HR decreased to 88 bpm during the second minute after which no difference between HR before and after calf removal could be seen (Hopster et al. 1995).

In a study made by Lefcourt et al. (1986) the HR peaked 5 s after stimuli (electrical shock) and returned to normal within 30 to 90s. Conversely no other physiological stress responses were found, although behavioural responses were severe and it was concluded that giving electrical shock was not a good way to evaluate stress. Electric shock did not seem to trigger the same responses as stress. The baseline HR was measured to 72.7±2.1 beats/min with an EKG monitor.

2.3.2 Blood pressure

The arterial blood pressure measures the pressure in the blood vessels during one cardiac cycle. The cardiac cycle is divided into systolic and diastolic phase and so is the blood pressure. During systole the heart contracts and maximum blood pressure is achieved, while during diastole the heart relaxes and blood fills the heart, hence the pressure in the blood vessels decreases. Arterial blood pressure can also be expressed as mean arterial blood pressure (MAP) and describes the average blood pressure in the vessels during one whole cardiac cycle (systole and diastole) (Sjaastad et al. 2003).

Blood pressure can be measured on animals with the same kind of equipment that is used on humans. The difference is that the sleeve is attached to the base of the tail instead of the arm or leg. This has been a usual approach for small laboratory mammals, like rats or mice for a long time. Already in 1978, Kvart showed that it could work well on large animals like horses as well. The biggest advantages is that it is non-invasive and do not require a lot of investment.

When testing on cows, Braslasu et al. (1999) used an electric oscillometric device, Omron R1, and attached it to the tail of 108 cows. After measuring each cow's tail blood pressure, averaged values of 102 mmHg systolic and 55.9 mmHg diastolic were achieved. Nevertheless most cows had a systolic value ranging from 90-99 mmHg and diastolic value of 40-49 mmHg. Moreover, they could not see any connection between blood pressure and age. Not surprisingly blood pressure in the tail increased when the cow was urinating or defecating. Cows exceeding 160 mmHg systolic and 100 mmHg diastolic where considered hypertensive after clinical examination. In 2011 the study was repeated on 150 Holstein and Brown cattle between 1 and 13 years old. The sleeve was attached at the base of the tail with the sensor at the ventral side measuring at the coccygeal artery. The systolic pressure was 103.75 ± 1.68 mmHg and diastolic pressure 56.48 ± 1.29 mmHg. The MAP was calculated to 72.26 ± 1.37 mmHg. Beside defecation and urination, they found that the noise caused by a tractor usually used to deliver feed caused a raise in blood pressure (Braslasu et al. 2011). In both studies the cows were awake and in standing position during measuring.

When evaluating stress during claw trimming blood pressure was also measured using an arterial catheter before and during the claw trimming. The blood pressure measured before treatment in the control group showed that systolic pressure was 136 ± 5 mmHg; diastolic pressure was 85.2 ± 5 mmHg and MAP was 107 ± 4 mmHg. When the cows were treated with saline solution before claw trimming a temporary increase in blood pressure was seen. In the xylazine treated group the decrease of MAP was more persistent and MAP was significantly lower compared to the control group (saline solution) (Rizk et al. 2012).

2.3.3 Respiratory rate

Respiratory rate (RR) is often measured to assess heat stress and is considered to be a stress response. The number of flank movements is counted during 1 min (Padilla et al. 2006, Rizk et al. 2012). RR in cows at normal housing temperature is significantly lower than in cows at heat stress. Cows in the control group, room temperature 18°C, had a RR of 38.8 breaths/min whereas cows in 28°C, (considered to be in heat stress) had 71.5 breaths/min (Padilla et al. 2006). Cows placed in lateral recumbency during claw trimming showed an increase in RR, arguably due to greater pressure towards the lungs from the rumen. RR was 26±3.0 breaths/min in the control group before claw trimming began and then increased to a maximum of 32±5.5 breaths/min. The groups treated with xylazine showed significant decrease in RR (minimum at 14 ±1.5 breaths/min) (Rizk et al. 2012).

2.4 Novel object test

Novel object test (NOT) is one of four classical fear or anxiety inducing tests; novel environment test, novel object test, surprise effect and food placed in an unfamiliar arena. NOT in cattle usually uses visual stimulus that are introduced by a human, or dropped from the ceiling. For example a green and white pyramid (0.4*0.4*0.4 m) was used as a novel object when conducting a novel object test on heifers. The object was placed in the pen before the animal was reintroduced to the pen in order not to elicit a response to the person placing the object. Unfortunately not much NOT studies have been done on cattle; hence how to interpret behaviours during NOT is uncertain. Still it was concluded that time spent away from the object is an expression of fear as well as vocalization and sniffing of the object expressed low levels of fear (Boissy and Bouissiou 1995).

Novel environment exposure and novel object test is also performed on pigs. The novel environment used in a test for pigs was a barren pen (5*5 m, concrete floor and hardboard walls) in which the pigs were kept for 5 min before the novel object test started. The novel object test was performed using a bucket lowered from the ceiling. The bucket was allowed to touch the floor in order to create some noise, but was then raised to 30 cm above the floor and left there for 5 min. During both test the pigs' behaviour was noted and blood samples were taken to measure serotonin levels. It was concluded that more behaviours and reactions were seen during the novel object test than in the novel environment exposure (Ursinus et al. 2013). The same approach as for the pigs was used on cattle in another study. The object used in this case was a blue plastic container (25*25*50 cm) and the novel environment was a 6*6 m pen with concrete floor and 2 m high wooden walls. After the plastic container was lowered with a rope to hit the floor, the container was left at 1 m above the floor in the centre of the pen for 10 min. During the test not only behaviour was recorded, but heart rate was continually recorded. Baseline heart rate was recorded in the home pen one week before the experiment. The heart rate during NOT was calculated to exceed the baseline by 25.0 ± 4.7 bpm. When the anxiolytic drug brotizolam was injected in higher doses (0.05-0.8 mg/100 kg bw) the response over baseline heart rate decreased, indicating that heart rate indeed is a good measurement of fear or anxiety level during NOT (Van Reenen at al. 2009).

It is protocol to test cattle alone , but still it is supposed that all herd animals experience stress when isolated and consequently isolation induces response (Herskin et al. 2004). Furthermore no relation between responses in home or novel environment has been seen, when exposed to novel stimuli. As a result the response recorded in novel environment does not have to reflect the response in the home environment (Munksgaard et al. 1997). Therefore Herskin et al. (2004) presented novel stimuli in the cows' home environment. The cows were housed in a tied up stall and no experimental cows were placed next to each other, hence the same novel stimuli could be used for all cows. Moreover, the cows were tested individually without being socially isolated. The novel stimuli used were novel object (white container 35*25*10 cm, filled with sand), unfamiliar food and an unfamiliar person. The result suggests that dairy cows give a greater response towards unfamiliar food or persons than towards novel objects. This is seen as increased exploration (e.g. sniffing) and reduced lying behaviour.

2.5 Cow Activity

Housing system and management influences weather the cow has enough time for different behaviours such as feeding, milking, eating, drinking and socialising. For this reason cows' lying and standing patterns can be used as a measurement of the cows' well-being and the comfort and planning of the stalls (Mattachini et al. 2011). Cows both inside and outdoors synchronise their behaviours. Inside this synchronisation is of course affected by feeding time, milking time and so on. Stoye et al. (2012) observed the synchronisation of lying behaviour in a herd of cattle on pasture and found that they had a more synchronised lying behaviour in evening and morning than in the middle of the day. The researchers propose that this could be due to dawn and dusk. The result also showed that the cows' behaviour is more correlated to the behaviour of the nearest neighbour than any random cow in the herd.

A small study by Phillips and Arab (1998) suggested that bullocks that wereheld in single pens show a small preference for standing and lying in lit areas if the bullock is alert. But if the bullock is passive and ruminates (standing or lying) this preference disappears and there seems to be no preference for light or dark. The authors also points out that due to the strong synchronisation of behaviour in herds of cattle, the preference to perform behaviour in light or dark may change in comparison with single held cattle.

Munksgaard et al. (2005) concluded that enough time for lying down (e.g. resting) was more important for the cows than eating and social contact regardless of state of lactation. When time available for eating decreased the rate of feed intake increased, minimising the loss in feed intake. This is not consistent with the founding that cows that have a higher milk yield spend less time lying down resting, probably because they need to spend more time feeding (Deming et al. 2013).

After investigating the lying and standing behaviour and pattern in thirteen AMS herds (data collected from 30 cows/herd) the average time lying was calculated to 10.8 ± 1.2 h/day, premilking standing duration 1.7 ± 0.6 h/day and postmilking standing duration was 1.25 ± 0.33 h/day (Deming et al. 2010). The authors concluded that daily lying time was positively associated with more space at the feed table and a higher frequency of feed push-ups. Comparable results was seen by Gomez and Cook (2010) that used video cameras to register the lying and standing pattern in 205 lactating cows in 16 loose housed herds in USA. The cows were either kept in stable with sand beds or with mattresses. The averages for the whole sample were calculated that the cows used 11.9 ± 2.4 h/day for lying down and resting. Time spent in alley (included time spent drinking) was 2.5 ± 1.5 h/day and time spent standing in the stalls were 2.7 ± 2.1 h/day. The time actually spent drinking were only 5-7 min/ day for each cow. However cows in stables with mattresses had a greater number of resting bouts but in shorter durations in comparison with the cows in stables with sand bedding and the authors concluded that the stall base type influences the resting behaviour.

Earlier researcher has been forced to use video recordings or visual observations to investigate the lying and standing pattern in cattle. Now animal activity monitoring sensors have been developed that not only is less time consuming, but also is a more objective measurement. Bewley at al. (2009) used these sensors to be able to investigate how the parameters milk yield, lactation stage and body score influences the time pent lying down. In average the cows spent 10.5 ± 2.07 h/day lying and 12.6 ± 1.97 h/day standing. The cows used were 77 Holstein-Friesian lactating cows. The cows were divided into group depending on body score, DIM (more or less than 150 days in milk) and kept in two different housing systems (parlour or AMS). The results indicated that the only factor affecting the lying behaviour was DIM.

3. Materials and methods

The trial was conducted at the Swedish Livestock Research centre, Lövsta and was approved by the Uppsala animal ethics committee. The trial lasted for four weeks, from the 7th of October to the 1st of November. The trial was conducted on four groups of dry dairy cows; each group was in the trial for four days.

The trial consisted of two experiments; 1) an obstacle course constructed of horse obstacles and 2) a novel object test (NOT). Throughout both experiments the cows' heart rate, respiratory rate and blood pressure were measured. During the trial the cows was also part of a behavioural study using the same experiments. The treatments used during the experiments were different light intensities with or without the presence of red light. The light intensities aimed for were 0, 5, 20 and 50 lux.

The experimental design used was a Latin Square. This meant that all the cows experienced all the light intensities but in different order so that the effect of day would be minimized. A Latin square was used in both experiments.

3.1 Animals and Housing

Twelve dry cows were included in the trial, two Holstein and 10 Swedish Red. They were chosen according to the criteria that they had to be dry since one week, have at least two weeks until parturition and have good hoof and leg health. If signs of bad claw and leg health were seen that cow had to be replaced during the trial. The cows were between 3 and 6 years old and had on average 46±10 days left to expected calving. In average the cows had been dried off since 70±25 days and was about to enter their 2±1 lactation. Individual cow specifics are available in appendix 1. The cows in the first two groups were taken directly from pasture to the trial, while the cows in the last two groups were housed indoors before being moved to the trial.

Three cows were moved every week to the stable and into single pens Monday afternoon in order to acclimatize them to the stable before the trial started the morning after (outline sketch of stable see figure 1). On Friday evening the cows were moved back to the herd. The pens were cleaned and bedding material refilled twice daily. The bedding material used was wooden shavings. Faeces were removed from the obstacle course between each run and the aisle leading up to the obstacle course was cleaned with a water hose at the end of the week.

The cows were feed silage *ad lib* during daytime but restricted during night time. Small amounts of concentrate were feed during and after the obstacle course in order to motivate them to complete the obstacle course. Free access to water was provided in the pens through a water cup.

The lights in the stable was turned off after last feeding and turned on at morning feeding. During the day only the light used in the trial was turned on. The stable had no day light inlet and during the night dim light was left on in accordance with the Swedish Animal Welfare regulations.

3.2 Experimental design

In the first test the cows were to navigate through an obstacle course at different light intensities. In the second test novel objects were introduced in to the single pen and the cow´s reaction was recorded. Throughout the trial the cows was also part of a behavioural study. During the obstacle course step rate, interactions with the environment, defecation, vocalization, knock down of or passing over obstacles was noted. In NOT time to contact with object, numbers of interactions with object and duration of the interactions was observed and noted.

The treatments are called 0, 5, 20 and 50 lux because this were the intensities aimed for (actual used intensities and total average are seen in table 1). These light intensities were tested both with and without presence of red light. Red light added approximately 0.2 ± 0.1 lux in the obstacle course and the NOT. The light was measured at a level of 80 cm from the floor at six points in the obstacle course and in the centre of the pen in NOT (see measuring points in figure 1).

Table 1. The average light intensities achieved during the tests. Presented as means and standard error.

	Obstacle course				Novel object test			
Group/Lux	0	5	20	50	0	5	20	50
1	0 ± 0.0	5 ± 1.0	20 ± 8.0	49 ± 30	0 ± 0.0	5 ± 0.2	20 ± 1.0	45 ± 2.0
2	0 ± 0.0	5 ± 1.0	19 ± 6.0	50 ± 25	0 ± 0.0	5 ± 0.3	20 ± 1.0	48 ± 2.0
3	0 ± 0.0	5 ± 1.0	19 ± 7.0	50 ± 26	0 ± 0.0	5 ± 0.2	20 ± 0.5	50 ± 0.3
4	0 ± 0.0	5 ± 1.0	21 ± 6.0	51 ± 27	0 ± 0.0	5 ± 0.0	21 ± 1.0	50 ± 1.0
Average	0 ± 0.0	5 ± 1.0	20 ± 1.0	50 ± 2.0	0 ± 0.0	5 ± 0.1	20 ± 0.5	48 ± 1.0

In order to achieve the different light intensities in the obstacle two ceiling lights (that had no manual switch off) were fully or partially covered. The lights in the ceiling were fluorescent light. In addition a building lamp (halogen light) could be redirected and thus be directed against the darkest parts of the obstacle course or the pens. For example to get 50 lux all ceiling lights had to be turned off, with the exception of the two lights that had no switch off. Due to this strategy the light was not evenly distributed along the obstacle course or in the pens. In order to achieve somewhat more evenly distributed light, light chains were used. They were attached alongside the obstacle course in height with the cows' head and above and alongside the front of the pens. The lights in the light chains were LED-lights. This was also the case in the light chains with red light, which were placed at the same position as the white light chains. Neither white nor red lights were controlled to be pure white or red light.

3.2.1 Obstacle course

Every day before the light intensity was set, measurements of blood pressure, heart rate and respiratory rate were performed in ordinary stable light (221±19 lux). After basal measurements were taken the cows were let through the obstacle course once in ordinary stable light. This obstacle course is called "familiar obstacle course" because the cows had had one test run to get accustomed with the obstacle course and the procedure of the test. The familiar obstacle course was always the first obstacle course for the day (figure 1). Thereafter the light intensity in the obstacle course was set and the cows were left in the pens for minimum 30 min to be habituated. The light intensity was controlled with a light meter 80 cm above the floor, at six points along the centre of the obstacle course when the light intensity was set, to get an average across the obstacle course of approximately the decided intensity (table 1).

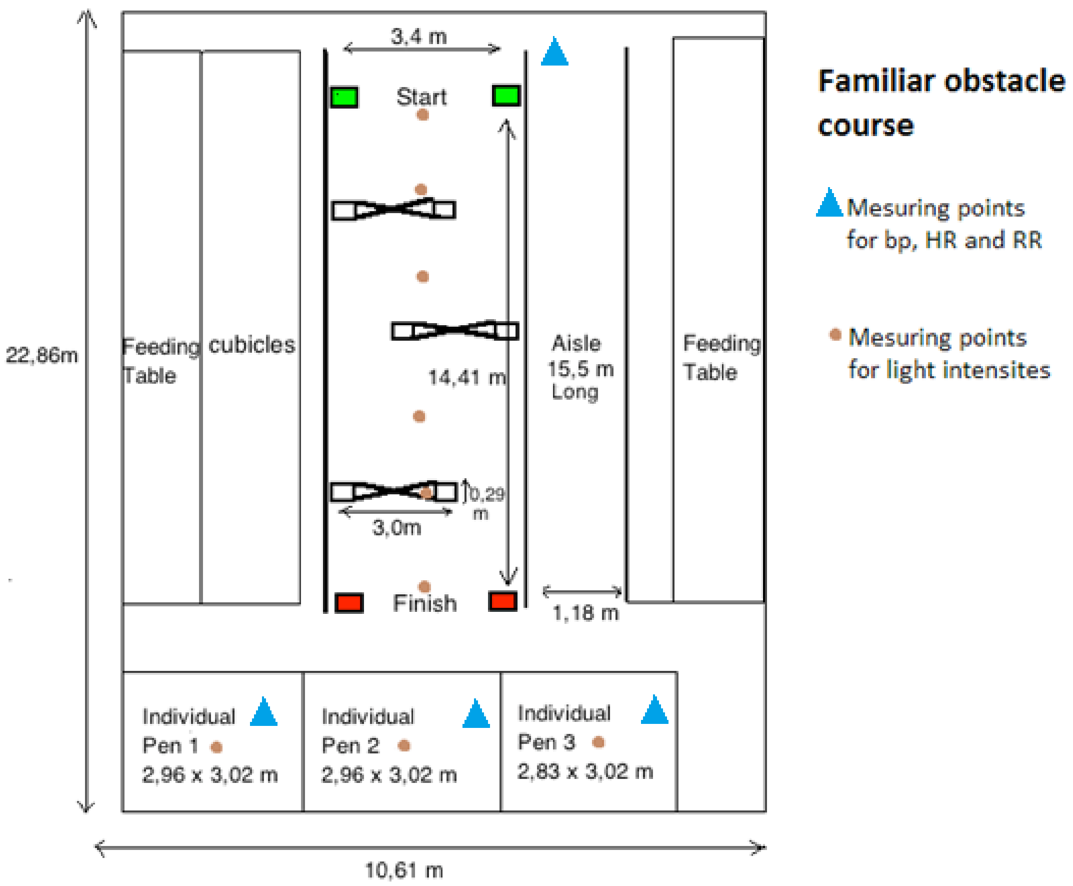

Figure 1. Illustration of the obstacle course (not drawn to scale).

After being habituated to the light intensity the cows were encouraged to walk through the familiar obstacle course twice, once in white light and once with additional red light. The order of the light was randomized on group level. Then the obstacle course was changed and the cows let through without a test run. However this time the obstacle was moved between white and red light to create a new obstacle course for each treatment. The changed obstacle courses are called "unfamiliar obstacle course" because the cows had never navigated through the specific obstacle course before the test. There were eight different unfamiliar obstacle courses (appendix 2). The unfamiliar obstacle courses were used in the same order every week, hence in different light intensities every week and in different light colour for different cows. Every cow passed through the obstacle course four times per light intensity: two times in familiar obstacle course, once in additional red light and once in white light, and two times in two different unfamiliar obstacle courses of which one were in white light and one in additional red light.

The reason for using familiar and unfamiliar obstacle course were to be able to see if any differences were due to learning the obstacle course rather than due to the treatments, and if so, be able to compensate for this. In practice the familiar obstacle course symbolises the cows' normal environment e.g. the way to the AMS in a free-stall. While the unfamiliar obstacle course would be a transfer to a new environment e.g. a dry cow moved from the milking group to a new stable or other change in environment e.g. new interior in the old stable. The order of white and red light in both familiar and unfamiliar obstacle course was also alternated to minimize the effect of learning the obstacle course.

Blood pressure and heart rate (HR) were measured with the help of a Vet High Definition Oscillometry monitor (HDO monitor) MD Equine (System + Beratung medVet GmbH Babenhausen, Germany). A cuff, similar to that used on humans, was attached at the base of the tail and the sampling was repeated until six values were obtained. The measurements were taken at three points; in the single pen before and after the obstacle course and in the aisle before the obstacle course (figure 1).

These measuring points were chosen in order to be able to seclude the rise in blood pressure and HR due to movement e.g. the walk in the aisle and in the obstacle course, from the rise due to the different treatments. The respiratory rate (RR) was taken at the same time by counting the numbers of flank movements in one minute.

The obstacle courses were constructed using white horse obstacles which consisted of rails and cavaletti blocks. All obstacles used were white to ensure that all the obstacles gave the same reaction, regardless of the colour of the light. The aisle was used to facilitate the transport of the cows to the starting line and the measurements before the obstacle course. When navigating through the obstacle course the cow walked toward the single pens with the remaining two cows in and furthermore a bucket with concentrate was used to actively call and encourage the cow to move. Also one person went behind the cow and encouraged her to move if standing still for longer period of time. At 0 lux infrared (IR) light and cameras were used in order to see the cows.

3.2.2 Novel object test

During the second test a foreign object was placed in the right corner of the pen by the test person. The object was left in the right corner of the pen for a period of 15 min. The same eight light treatments as in the first experiment were used. Eight different objects were used (appendix 3); a new object for every treatment, hence all cows experienced all objects. However in what order and in what light colour and intensity was randomized between cows. The objects were a pink box, a black umbrella, a green swimming ring, a multicoloured beach ball, a multicoloured pinwheel, an orange cone, a red shopping bag and a blue IKEA bag.

The light intensities were controlled in the pens using a light meter, measuring 80 cm from the floor in the centre of the pen (see figure 1). Blood pressure, HR and RR were measured before a novel object was introduced into the pen. When the object was introduced to the pen the cow had to be standing and the cow's head had to be inside the pen. If the cow was lying down or standing with the head outside the pen in the trough, a test person encouraged the cow to stand up or move before the test started. When the object was removed measurements of blood pressure, HR and RR were taken once again. The test was repeated twice per day for each cow; once in white light and once in red light. The order of white and red light was randomized at cow level. Between each test there was at least 15 min recovery for each cow.

3.2.3 Activity measurement

Starting week two, the cows were fitted with activity meters (IceTag sensor for cattle, IceRobotics, Edinburgh, Scotland UK) that measured the activity of the cow; whether they stand, walk or lie down. The activity meter was attached to the left hind leg. Week one and two the activity meter was attached Monday evening and the last week on Tuesday morning and was removed Friday evening. Unfortunately one of the IceTag sensors malfunctioned during the second and last week, therefore records were only obtained from six cows, two cows from each week.

3.3 Data handling

Data from the measuring of blood pressure, HR, RR and the activity meters were statistically analysed in SAS 9.3 (Statistical Analysis System, Cary USA). The model used in SAS was the Mixed model with the estimation method Restricted Maximun Likelihood (REML).

The parameter used for blood pressure was MAP (medial arterial pressure) rather than systolic and diastolic arterial pressure which is both closely connected to MAP. When analysing the results all parameters were analysed as differential. Two different differentials were used. The first one called "prepost" during analysis, describes the difference between the measurement done in the pen just before the experiment and the measurement done back in the pen or after the experiment is completed. Therefore showing the difference created due to the test. The second differential, only used for the obstacle course was called “durpost” during analysis. It describes the difference between the measurement done in the aisle before the obstacle course and the measurement conducted back in the pen afterwards.

Variables used for the different experiments during the statistical analysis can be seen in table 3. The most important effects tested for being light intensity and light colour, thus the most important interaction being light intensity*light colour, but both 2- and 3-way interactions were tested. Cow was considered to be a fix factor during the analyses. The obstacle course was described and analysed both as familiar and unfamiliar and as nine different designs.

Table 2. The variables used during the statistical analyse

	In common	Obstacle course (ob.c)	NOT
Variables	light intensities colour of light directly from pasture or not batch no. of days in the experiment	different obstacle courses runs per day totals no. of runs	different objects no. tests per day total no. tests pen

The data from the activity meters was also analysed in SAS. However the only treatment considered was light intensity. The variable used was the same as for obstacle course and NOT (in common, table 2). The parameters used were the sum of minutes and the proportion time spent standing, lying or changing position.

The data is presented as LSmeans and standard errors. The significance level used was p=0.05.

4. Results

4.1 Heart rate

The difference in heart rate between before and after the tests, measured in the pen, was not significantly affected by the different treatments in neither obstacle course (light intensity p=0.7921, with red light vs. only white light p=0.3786) nor NOT (light intensity p=0.6993, with red light vs. only white light p=0.7991) (table 3).

Table 3. LSMeans for the heart rate (HR) before the test and the HR after the test, both measured in pen and average difference in HR. The difference in HR was calculated as HR-value after the test minus HR-value before the test. The LSMeans are presented with standard error.

	Obstacle course			**Novel Object Test**		
Lux	Mean values before	Mean values after	Average difference	Mean values before	Mean values after	Average difference
0	88 ± 12	87 ± 12	1 ± 1	96 ± 12	96 ± 11	-4 ± 3
5	90 ± 12	89 ± 12	0 ± 1	93 ± 11	91 ± 9	-3 ± 3
20	87 ± 13	86 ± 12	–1 ± 1	94 ± 13	90 ± 9	0 ± 3
50	88 ± 15	88 ± 17	1 ± 1	95 ± 95	95± 13	-2 ± 3
Colour of light						
Only White	88 ± 10	87 ± 9	0 ± 1	94 ± 10	92 ± 11	-2 ± 2
Additional Red	88 ± 9	88 ± 9	1 ± 1	95 ± 13	94 ± 10	-3 ± 2

A significant effect on the difference in HR was seen as a result of familiar or unfamiliar obstacle course. The difference in HR between the measurement done before the obstacle course in the pen and after the obstacle course back in the pen, was negative in the familiar obstacle course but positive in the unfamiliar (-2.4 and 2.6 respectively, p=0.0477).

The interaction between light intensity and presence or absence of additional red light were not significant for the obstacle course (p=0.1040) and showed a tendency in NOT (p=0.0691). 0 lux with additional red light showed a tendency to give a bigger difference in HR compared to 0 lux with no additional light (p=0.0855) during NOT (figure 2).

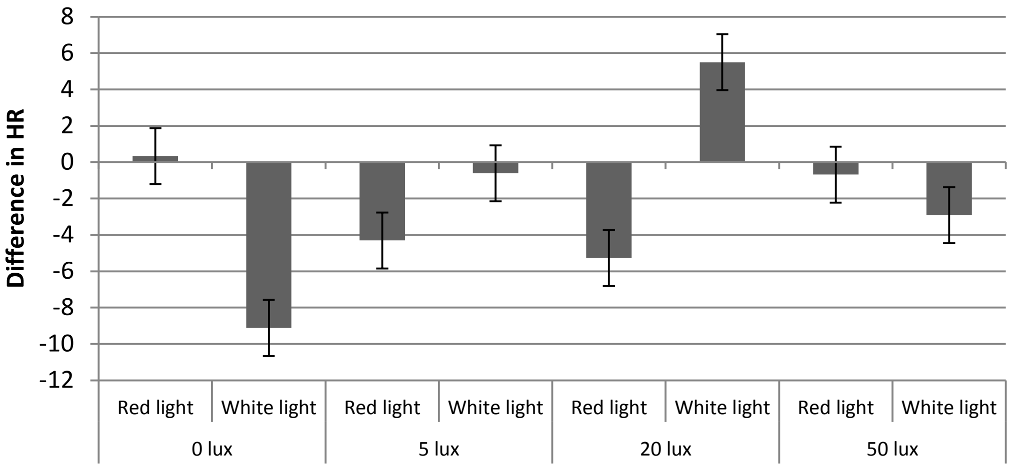

Figure 2. The difference in heart rate (HR) for the novel object test at different light intensities with additional red light or only white light. The difference in HR was calculated as the HR-value measured after minus the HR-value measured before the test. Values are presented as LSMeans, error bars display the standard error.

In the NOT the umbrella was the object that caused the biggest positive difference in HR between the measurement done before the test and after the test. It showed a significant difference in comparison with the red shopping bag (p=0.0496), the orange cone (p=0.0163), the pink box (p=0.0041), the beach ball (p=0.0027), the IKEA bag (p=0.0146) and swimming ring (p=0.0458).

4.2 Blood pressure

The difference in MAP before and after the tests measured in the pen was not significantly affected by the different treatments in NOT (light intensity p=0.7857, with red light vs. only white light p=0.8251) and the same was true for the light intensity in the obstacle course (p=0.8350). Nevertheless there was a tendency towards lower MAP in the presence of additional red light compared to only white light (p=0.0833) in the obstacle course (table 4).

Table 4. LSMeans for mean arterial pressure (MAP) before the test and MAP after the test, both measured in pen and average difference in MAP presented with standard errors. The difference in MAP was calculated as MAP-value after the test minus MAP-value before the test

	Obstacle course			**Novel Object Test**		
Lux	Mean values before	Mean values after	Average difference	Mean values before	Mean values after	Average difference
0	85 ± 9	85 ± 8	-1 ± 2	89 ± 12	85 ± 12	-4 ± 3
5	84 ± 10	84 ± 10	1 ± 2	84 ± 14	81 ± 14	-3 ± 3
20	87 ± 10	84 ± 9	-1 ± 2	85 ± 12	85 ± 12	-0 ± 3
50	84 ± 10	84 ± 9	1 ± 2	85 ± 13	83 ± 13	-1 ± 3
Colour of light						
Only White	84 ±14	85 ± 13	2 ± 2	86 ±13	83 ± 13	-2 ± 2
Additional Red	86 ± 12	83 ± 14	-2 ± 2	86 ±13	84 ± 10	-2 ±2

Some differences could be seen in MAP due to different obstacle courses (figure 3). Obstacle course a (familiar obstacle course) gave a positive difference in MAP between before and after the obstacle course, before measured in the aisle and after measured in pen, and differed significantly from obstacle course h (p=0.0440), that gave a negative difference in MAP.

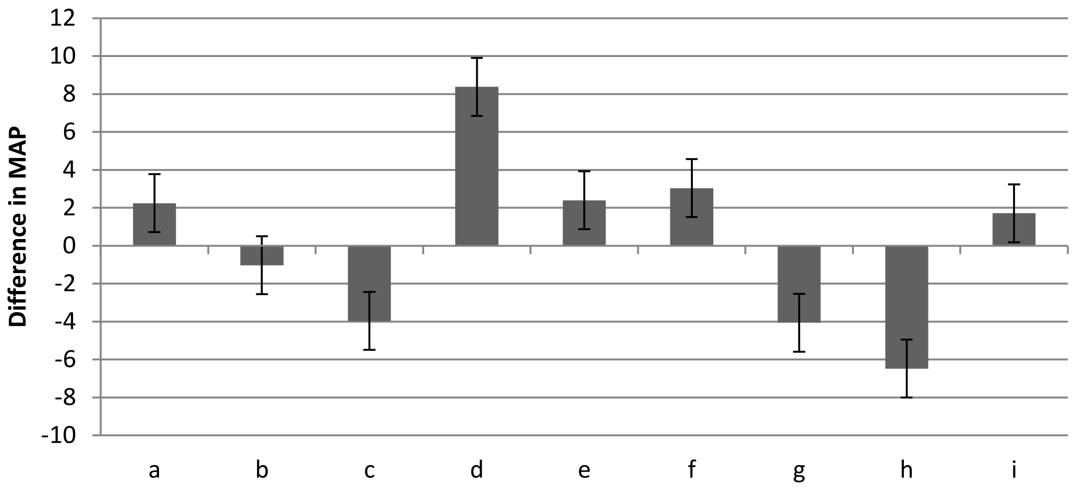

Figure 3. Average difference in mean arterial pressure (MAP), due to different obstacle courses. The difference in MAP was calculated as the after value (measured in the pen) minus the before value (measured in the aisle). Values are presented as LSMeans, error bars display the standard error.

In the interaction between light intensity and the presence or absence of red light, a tendency to affect the difference in MAP was seen in NOT (p=0.0881). 0 lux with red light showed a tendency to differ from 0 lux with no red light (p=0.0915) (figure 4).

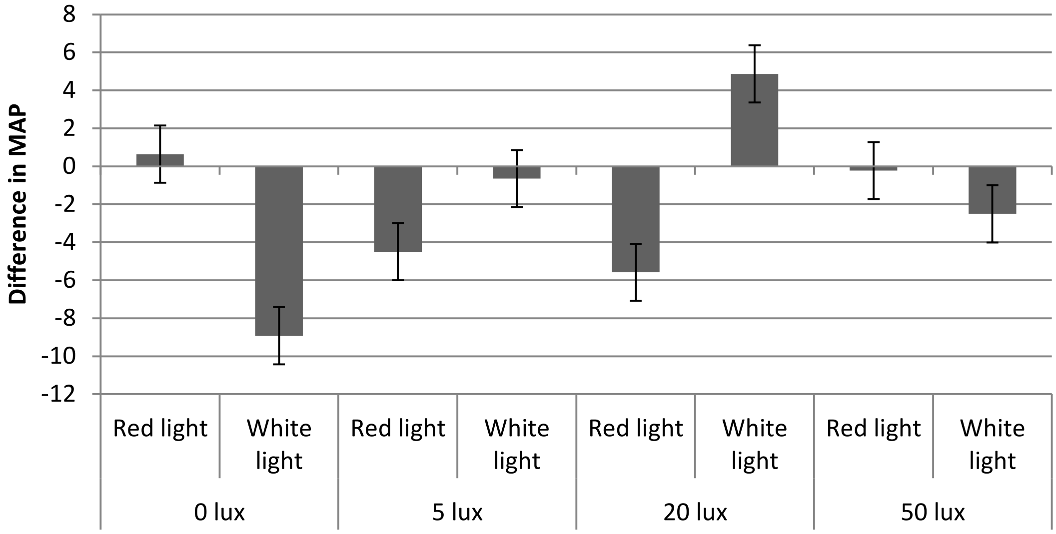

Figure 4. The difference in mean arterial pressure (MAP) at different light intensities with additional red light or only white light during the novel object test. The difference in MAP was calculated as MAP-value after the test minus MAP-value before the test, both measured in the pen. Values are presented as LSMeans, error bars display the standard error.

In the NOT the umbrella was the object that caused the biggest positive difference in MAP as well, between the measurement done before the test and after the test. It showed a significant difference in comparison with red shopping bag in white light (p=0.0237), the orange cone in red light (p=0.0094) and in white light (p=0.0475) and the pink box in red light (p=0.0011).

Moreover the pink box in red light shows a tendency towards greater difference in MAP than the umbrella in white light (p=0.0613), beach ball in white light (p=0.0848) and pinwheel both in white (p=0.0927) and red light (p=0.0927) (figure 5).

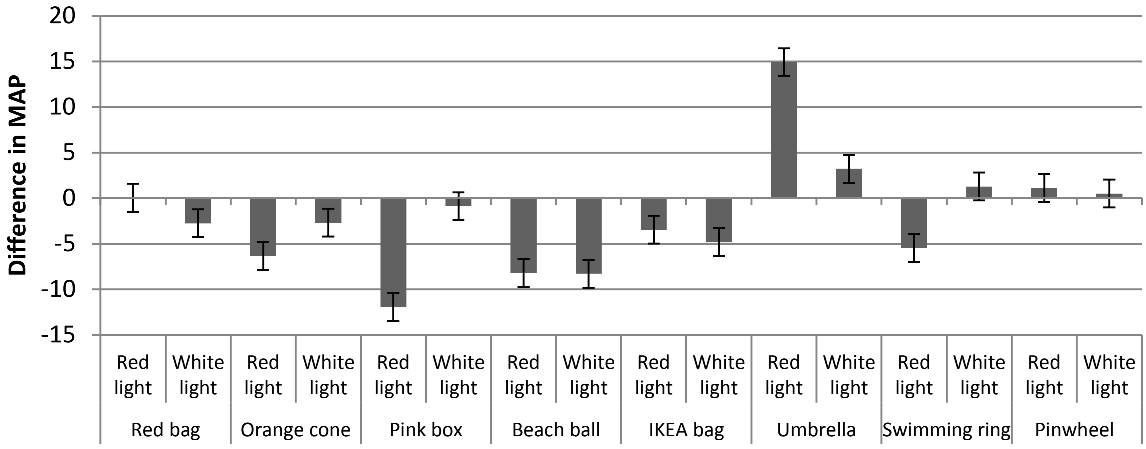

Figure 5. The difference in mean arterial pressure (MAP) for all objects with or without red light during the novel object test. The difference in MAP was calculated as MAP-value before test minus MAP-value after test, both measured in pen. Values are presented as LSMeans, error bars display the standard error.

4.3 Respiratory rate

The difference in RR was significantly affected in the obstacle course depending on red or white light (p=0.0063); the difference in RR between before and after the test was negative and smaller in white light than in red light, which was positive (table 5). Nevertheless when using the measurement in the aisle instead of in the pen before the obstacle course, there were no longer any differences in RR due to presence of red light could be seen.

The difference in RR between measurements done before and after the test was not affected by the different light intensities in the obstacle course (p=0.9286), however this could be seen in the NOT (p=0.0038). The difference in RR was significantly higher in 0 lux than in 5 (p=0.0045), 20 (p=0.0006) and 50 lux (p=0.0161) during the NOT. Likewise the difference in RR was significantly affected by the presence or absence of red light (p=0.0463) in NOT as well (table 5).

Table 5. LSMeans for respiratory rate (RR) in the pen before the test and RR after the test also in pen and the difference in RR calculated as RR-value after the test minus RR-value before the test. The figures are presented with standard errors

	Obstacle course			Novel Object Test		
Lux	Mean values before	Mean values after	Average difference	Mean values before	Mean values after	Average difference
0	27 ± 6	28 ± 7	1 ± 1	26 ± 7	30 ± 7	3 ± 1[a]
5	26 ± 4	27 ± 4	1 ± 1	31 ± 6	29 ± 8	-1 ± 1[b]
20	27 ± 6	27 ± 6	0 ± 1	28 ± 4	26 ± 6	-2 ± 1[b]
50	29 ± 6	29 ± 7	0 ± 1	31 ± 5	30 ± 6	0 ± 1[b]
Colour of light						
Only White	29 ± 6	27 ± 6	– 1 ± 1[a]	29 ± 6	29 ± 7	1 ± 1[a]
Additional Red	27 ± 6	28 ± 7	2 ± 1[b]	29 ± 6	28 ± 7	– 1 ± 1[b]

[a,b]Superscript letters indicate significant (p≤0.05) differences between treatments within column

An effect of the interaction between light intensity and presence of red light on the difference in RR could be seen in the obstacle course (p=0.0220) but not in NOT (p=0.1407). The most interesting difference due to the interactions in obstacle course was between 0 lux with red light and 0 lux with no light (p=0.0001) (figure 10). Both 0 lux with or without additional red light also significantly differed (p≤0.05) or showed a tendency (p<0.1) to differ from all other light intensities (figure 6).

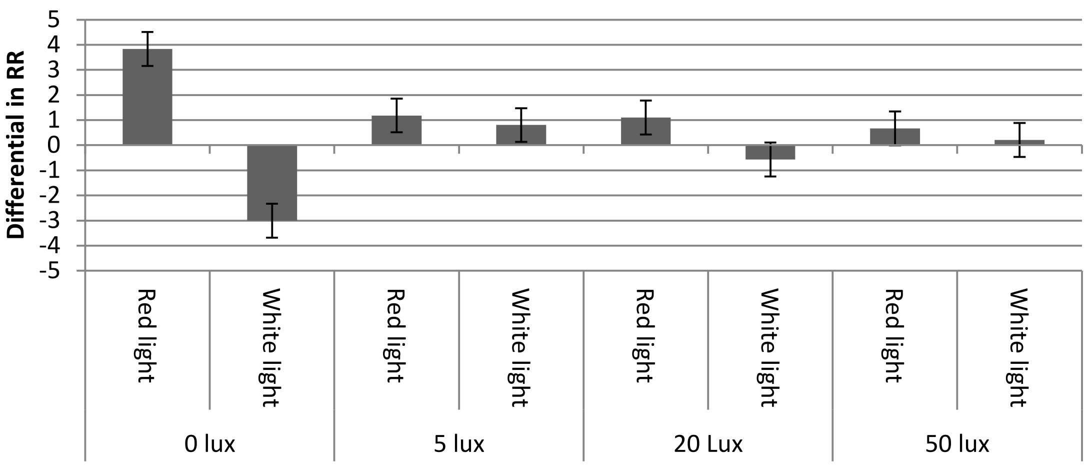

Figure 6. The difference in respiratory rate (RR) for the different light intensities with or without additional red light during the obstacle course. The difference in RR was calculated as RR-value after the test minus RR-value before the test. All measurements were done in the pen. Values are presented as LSMeans, error bars display the standard error.

The orange cone used in NOT gave a greater difference in RR than other objects. The orange cone gave significantly greater difference in RR than the red shopping bag (p=0.0269), the pink box (p=0.0202) and the swimming ring (p=0.0235) (figure 7).

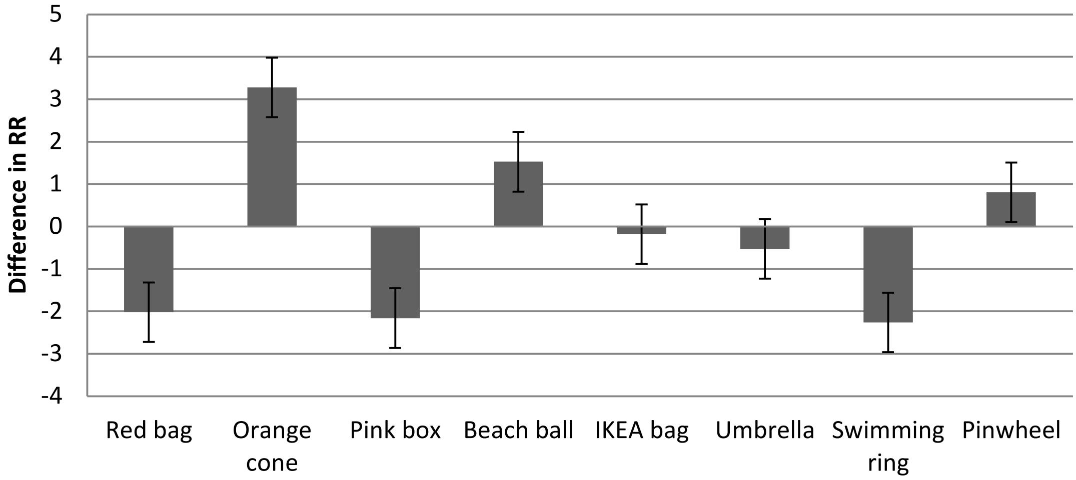

Figure 7. The difference in respiratory rate (RR) for the different objects in the novel object test. The difference in RR was calculated as RR-value after the test minus RR-value before the test. All measurements were done in the pen. Values are presented as LSMeans, error bars display the standard error.

4.4 Activity

The cows spent in average 11.5±2.1 h/day lying down. A bigger percentage of time was spent lying in 50 lux than in any other light intensity (figure 8). Still only a tendency was seen was in comparison between percentage of time spent lying in 50 and 20 lux (p=0.07).

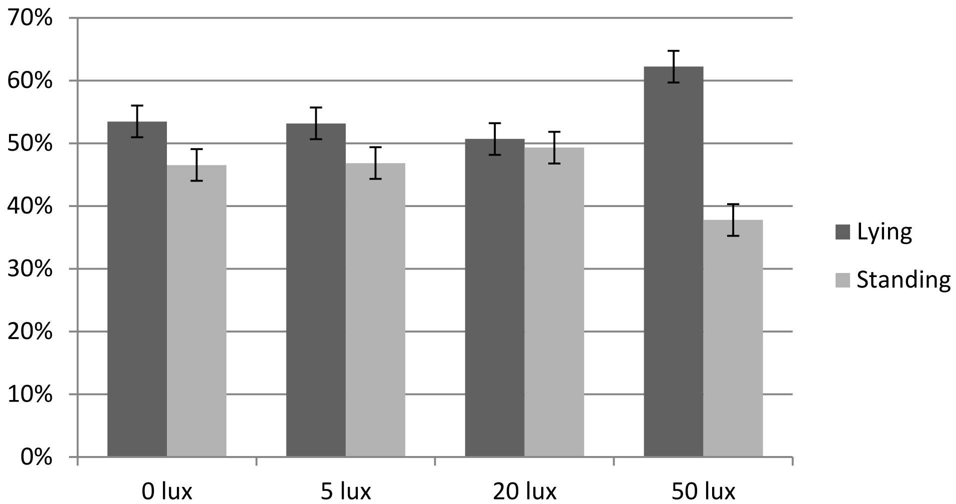

Figure 8. The average percentage of time lying and standing in different light intensities. Values are presented as LSMeans, error bars display the standard error.

5. Discussion

Heart rate and blood pressure were not affected by the treatments neither in obstacle course nor NOT. However blood pressure showed some tendency to be lower in red light than in white only. Respiratory rate was affected by the presence of red light in both obstacle course and NOT, but no differences due to light intensities could be seen in the obstacle course. Conversely RR was higher in 0 lux than in any other light intensity in the NOT.

The HR was affected by whether the cow went through a familiar or unfamiliar obstacle course. The HR decreased between before and after test in the familiar obstacle course and increased in the unfamiliar obstacle course. This suggests that the cows do learn their environment and that their HR get more affected by an unfamiliar or changed environment. It also suggests that cows may be more stressed by new environments. In this situation the cow is in a new environment (unfamiliar obstacle course) which is stressful but not surrounded by new group members which could attack or bully her. The light could be turned on in order to let the cow explore the new environment, but can still be kept dim in order to facilitate her to explore the environment with the help of her nose. To have complete darkness may risk her to get anxious and may consequently start to run, kick or in other way risk hurting herself or others. For example one of the cows during week two tried to jump over the walls of the pen while vocalizing loudly, several times the first day which had 0 lux as treatment. This could be interpreted as anxiety, fear or stress and an aversion towards darkness. Still the severe reaction of this particular cow may not have been due to aversion to darkness but as a result of isolation. The other cows did not respond to her vocalizing and the darkness may have prevented her from perceiving the others. It is likely that the most stressful factor in this case was the isolation rather than the darkness, but the isolation was perhaps reinforced by the darkness.

In the obstacle course MAP was affected differently by different obstacle courses. This could indicate that the obstacle courses differed in difficulty. If some off the obstacle courses were more difficult to navigate through, the parameters might have been more affected during those. The familiar obstacle course made MAP significantly higher than obstacle course h (outline in appendix 2), this indicates that MAP increased during the test compared with before the test. In figure 3 it is also seen that several other obstacle courses gave lower difference between MAP before and after the test than the familiar obstacle course. This is not in line with the hypothesis that unfamiliar obstacle courses were more stressful than the familiar. However if the familiar obstacle course is perceived as more difficult by the cows than some of the unfamiliar obstacle courses this could interfere with the fact that the cows recognize the environment or not.

The fact that the familiar obstacle course always was carried out before unfamiliar obstacle course regardless of treatment, day, week, cow or group, could have affected the result. All the cows did the familiar obstacle course first before the obstacle course was rebuilt and the run through the unfamiliar obstacle course started. This meant that the cows had some relation to what was going to happen when the measurements before the unfamiliar was taken and that could have affected the measurements e.g. if the cow got stressed by being put through the same procedure again, or calmed down because she recognized the situation. Similar reaction can be discussed about the measurement taken before the familiar obstacle course; especially the first day of the test week there was a novelty of the test that could have affected the measurements.

In the NOT the HR seems to be affected in different extent by 0 lux with the presence of red light than without any light and the same tendency is seen for MAP during NOT. 0 lux with additional red light shows a positive difference which means that HR and MAP has increased during NOT, while 0 lux only shows a negative difference, hence meaning that HR and MAP has decreased during NOT. During the obstacle course RR also showed differences between 0 lux with red light, where the RR increased, and without any light, where the RR decreased. All parameters increased in the presence of red light, this suggests that red light can be detected by the cows because there were no other light sources during 0 lux. RR was highest in 0 lux with red light and lowest in 0 lux without any light, this would further strengthen the belief that cows do detect red light. Since the parameters increases the cows seems to be more stressed in red light than in darkness which could mean that the cows do see in red but not in darkness, or it could suggest that red is a stressful colour for the cows. Since no parameters besides RR were significantly affected by different light intensities it is not likely that the cows are totally blind in 0 lux. If the cows were virtually blind in 0 lux, there should been seen a difference between 0 lux and the other light intensities. As indicated above there is such a difference in RR, during NOT 0 lux gives a significantly higher difference than the other light intensities. However it feels safe to state that in this study the cows were not generally stressed by darkness.

The difference in RR was significantly higher in 0 lux than any of the other light intensities during NOT, the same is not seen in obstacle course. Also there was a difference between RR in red vs. only white light. RR increased in only white light but decreased in red light during NOT, the opposite is true for the obstacle course. Why the RR would decrease in red light during NOT but increase during obstacle course is not clear, but it could be that the RR was affected by the movement of the cow in the obstacle course. The significance disappeared in the obstacle course if the difference in RR was calculated by the before value measured in the aisle instead of the value measured before in the pen. This supports that the RR would be affected by the movement of the cow and hence could mask the effect on the RR by the test itself.

The additional red light did not affect the light intensity very much (besides in 0 lux with additional red light) but some results indicates that different colours and wavelengths affects the cows differently. Phillips and Lomas (2001) could for example see that calves were more active during NOT in red light than in blue or green light. To detect the colour red could very well be important for cattle in order to detect the swelling and redness of the vulva during oestrus or to the attack of a predator drawing blood. In these cases smell most certainly is the most important sense, but the more senses used insures a more secure result. Uetake and Kudo (1994) propose that red is the colour that is hardest to perceive for cattle (in comparison with white and green light), as in this study the light used were not thought to be pure and the wavelength of the coloured light was not known.

In opposite to the hypothesis, HR and MAP decreased instead of increasing during NOT. This suggests that the cows were not only, not stressed by the NOT but maybe they also calmed down. The NOT was always after completing the obstacle course, therefore always in the afternoon. Also the stable was much quieter when no cow was in the maze and all humans were sitting still and being quiet. This may have had a greater soothing affect on the cows than the stress response elicited by the test.

RR is mainly used as an indicator of heat stress (Rizk et al. 2012, Padilla et al. 2006) and may be a way to cool down the body rather than an actual sign of stress or fear. This is supported by the result that the effect of presence of red light on RR disappears when the results are calculated with the before value measured in the aisle rather than in the pen. Also all cows but one was pregnant, as a result their RR could have been affected. The size of the calf and the number of calves determines how much room that is left in the body cavity for the lungs to expand (Rizk et al. 2012).This was in some way counteracted by only comparing differences in RR between cows instead of comparing actual values. In this way the cows RR after the experiment was compared to its own RR before the experiment, thus it did not matter if the cows had different baselines compared to other cows, only the differentials within each cow were compared between cows.

It was very difficult to count the number of flank movements in order to get the respiratory rate. In most cases it was not enough to look at the cow but physical contact was needed as well in order to feel the movements. But even with physical contact it was hard to count, especially if the cow was eating and if further studies are conducted it is recommended to remove the feed during the counting of flank movements. It was particularly hard to observe the RR in the darker light intensities and complete darkness and had to be base almost solely on physical contact. The physical contact may also have influenced the result in itself. Schmeid et al. (2008) could see that cows that were used to physical contact with humans (stroking) lowered their HR during treatment (stroking of different regions of the body), while the cows not used to physical contact with humans had an increased HR during treatment.

The baseline values attained for HR was in average for all cows 86 ± 8.6 bpm which can be seen as within the normal range as suggested by Hopster et al. (2002) and supported by the findings of several others (Lefcourt et al. 1986, Schmeid et al. 2008, Lefcourt et al. 1999 and Rizk et al. 2012). The same average baseline value were 66 ± 10.7 mmHG for MAP, 65 ± 13.7 mmHG for diastolic pressure and 114 ± 14.7 mmHG for systolic pressure. Brauslasu et al. (1999 and 2011) used the same method and showed somewhat lower but similar results. The cattle used in that study had a more varying age and this may have affected their average as blood pressure changes slightly with age. On the other hand the measurements done before treatment in the study conducted by Rizk et al. (2012), were a little bit higher than in this study and also considered to be normal values. This indicates that the cows in this study had a fairly normal blood pressure during the baseline measurements. The average RR during baseline measurement for all cows were 27 ± 6 breaths/min this is consistent with the measurements conducted by Rizk et al. (2012) but lower than the measurements done by Padilla et al. (2006).

The cow that was not pregnant (id. 1590) stood out regarding MAP. Her blood pressure was in general higher than remaining cows, which was were interesting since she differed from the other cows. However no valid explanation was found.

During this study the baseline levels for HR, RR and MAP seems to be within the normal range. Despite this not much significant results are found due to treatments. This gives reasons to think that HR, RR and blood pressure are not so good parameters to use to investigate the stress and physiological changes in cows due to different light intensities or presence of red light. However this is a small study and more variations and differences may have been seen if a bigger group of animals were tested. It is not surprising that the cows would not be stressed by low light intensities since it is a natural part of the day, but this does not mean that light intensities does not affect the cows physiology and behaviour. In early planning of this study the intent were to select saliva for cortisol analysis, this later proved to not be possible. However cortisol may still be an interesting hormone to look into when investigating stress response due to different light intensities.

The activity meters recorded that the cows in average used 11.5 ± 2.1 h/day lying down during this study. This is in line with the results published by Deming et al. (2013) and Gomez and Cook (2010) 10.8 ± 1.2 and 11.9 ± 2.4 h/day, respectively. Bewley et al. (2009) used the same sensors as in this study and found that the cows in their study used 10.5 ± 2.07 h/day lying down, further supporting that the measurements in this study are within the normal range. Dairy cows use roughly 50-55% of the day for lying down (personal message Anders Herlin, 2013). This seems to be in line with the results in this study (figure 8). No matter what light intensity used the cows spend approximately 50% of their time lying down, besides in 50 lux were the cows seems to lay down for around 60% of the time. Still the difference is not significant. The motivation for cows to lie down is great and if denied signs off discomfort (e.g. leg stamping, shifting their weight between legs) and eventually behavioural disorders are seen (Cooper et al. 2008). Hence the goal from an animal welfare perspective should be to create an environment that supports the cows need for lying down.

The results from the activity meters show a tendency that a bigger proportion of time was spent lying down in 50 lux than in 20 lux. This was not expected, the hypothesis was that the cows would lay down more in the lower light intensities. Still one explanation could be that the cows feel more secure in 50 lux and therefore more easily lies down. In 50 lux the results suggest that the cows have no trouble to distinguish their surroundings, thus feel that they can detect danger in time even though they are lying down. This explanation would also be valid if they stood more in 0 lux; they feel insecure because they do not believe that they can detect danger in time. However in this study no difference between 0 lux and the other light intensities were seen. No significant difference in the percentage of time spent changing position was seen either. This means that the cows, even though they may lay down for a greater time of the day in 50 lux, they have equally many standing and lying bouts as in all other light intensities. The result may be different if a herd is monitored, in these single pens the cows cannot depend on any other cows keeping watch. Also in this study only six cows were successfully fitted with the activity meters and therefore the result may be a somewhat unreliable. However it shows that activity meters could be a useful tool to measure cows' comfort in different light intensities. Because we did not note the time when using red light every day we could not test for differences in activity due to red or white light.

During the NOT the umbrella seemed to give a bigger response in HR than all other objects beside the pinwheel, and gave an exceptionally increase in MAP during the test when put in red light. This may be due to the fact that the umbrella was black and when placed in the corner, which is somewhat darker than the surrounding, proved harder to detect for the cows. Also the cows in pen 2 and 3 often went to drink water from the water cup and discovered the object then. When drinking the cows often drops water all around splashing on the object beneath, this makes a louder noise when hitting the umbrella than any other object. This sudden noise might be the reason for the great reaction compared to the other objects. It is important to notice that no significant differences due to pen were seen despite the water cups being in different corners in pen 2 and 3 compared to pen 1. Why the umbrella gave a higher response in red light than in white light is not clear. The red light did not increase the light intensity to any substantial extent in any of the treatments besides 0 lux, where the different with and without red light of course were vast, but maybe the red light somehow interfered with the colours of the umbrella (black and a small amount of pink). And as mentioned earlier red may be an activating colour for cattle.

When looking at MAP during NOT, the pink box also showed some difference compared with the other objects, at least in red light. Because the pink box contains the colour red it could be difficult for the cows to distinguish the pink box in red light. The other objects that also contained the colour red (orange cone and red shopping bag) showed the same tendency toward lowered MAP as the pink box. As discussed before the objects may be hard for the cows to see these objects due to their colour. Another likely explanation is that the pink box is too similar to other containers in the home environment of the cows and therefore no stress response is elicited due to the box.

Furthermore when analysing RR for NOT another object stood out; the orange cone. Probably it was the reflexes that made it easier to see than the red shopping bag and the pink box. What colours that are distinguishable to cows are not fully agreed upon (Sjaastad et al. 2003, Munkgaard et al. 1996, Phillips and Lomas 2001, Dabrowska et al. 1981) and more research is required to fully understand the cows' view of the world. But these results do seem to support that cows can perceive red light and red colour, since this would explain why different coloured objects in different coloured objects elicit these responses.

The fact that different objects gave different results regardless of light intensity or the presence of red light shows that other factors interacted with the results. As mentioned above one such factor is probably the colour of the objects. Initial the objects were chosen with regard to both their colours and the likelihood that the cows had seen the objects in any other situations. The intention was to see if the interaction between the colour of the object and the colour of the light used in the barn affected if their visibility for the cows. The results point toward that such an interaction exists, but because all objects were used in different treatments for different cows and so few tests were done it is hard to see the details of the connections. Because the sample of cows used was so small it had been better to use objects in the same colour to get a clearer view on which factors that had the biggest impact on the results.

The decision to only use white obstacles in the obstacle course differs from the choice made in NOT. During the obstacle course the point was to see whether they could navigate through the obstacle course in different light intensities and if there were any differences due to the presence of red light. To use obstacles with different colours would have interfered and it would have been difficult to separate cause and effect. Especially since we used the same obstacles in all obstacle courses and moved them around to achieve unfamiliar obstacle courses.

Earlier researchers have either put the object into the pen when the animals were not in the pen (Boissy and Bouissiou 1995) or lowered the object from the ceiling, hitting the floor and causing a noise before being left hanging in the pen (Ursinus et al. 2013, Van Reenen at al. 2009). During NOT in the current study the object was always placed in the right corner by a test person. This was mainly to ensure that the object was placed in the same position every time, also this alarmed the cows on the fact that something was happening, similar to the noise made by lowering the object from the ceiling but surely not as aggressive. Perhaps it would have been better to place the object in the centre of the pen, the same place as the light intensity were measured, because the corners were generally darker than the rest of the pen. On the other hand the pens were not that big and the cow could have been "forced" to interact with the object even if the cow rather would have avoided the object completely. The placement of the object in the corner ensured that the cows could choose to not interact with the object. As stated earlier there were no significant differences due to pen in NOT even though two of the pens had the water cup in the corner used to place the object. For these reasons it is concluded that to put the object in the same corner every time was the right decision in this particular study. One way to avoid the cows to be "forced" to interact with the object but still be able to put the object in the centre of the pen would be to use a bigger pen. To lower the objects from the ceiling would have minimized the interaction with people and in a different situation would there for have been preferred.

In 0 lux without the presence of red light it was not possible to see the cows in the pen or in the obstacle course. This meant that one person always had to be looking at the computer through the cameras to see the cows. As a consequence only one person was available to herd and motivate the cow through the obstacle course. This could have an effect on the results in 0 lux; if the cows stood still more in the obstacle course and did not walk as fast through, then the HR, blood pressure and RR may be lower than they would be otherwise as a result. In this study no significant results were seen as a result of different light intensities for HR, MAP or RR. It is possible that this could be due to effects counteracting each other and in that way hiding the true effect of darkness.

To be able to see anything even in the computer, IR lights were used in 0 lux in order to be able to record the behaviours in NOT and the movements in the obstacle course. Today no evidence suggests that cows can detect IR, partly because the notion is that no mammals detect IR light (Sjaastad etl al. 2003). It is important to recognize that if this is not the case, the treatment called 0 lux would not appear dark at all to them. That would in such case explain way there were no differences between light intensities, however this seems improbable. IR is generally said to have wavelengths above 700nm (Sjaastadt et al. 2003) and Phillips and Lomas (2001) calculated that the top limit for the calves vision were 620 nm. This would suggest that the IR lights posed no problem for the execution of the test.

Cows do in greater extent rely on their sense of smell and hearing when vision is impaired. To make the cows motivated to go through the obstacle course, a bucket of concentrate was used. The handler shook the bucket in front of the cow in order to make the cow aware of this reward. This helped the cows in some extended to orientate themselves in the obstacle course with the help of other senses than vision. But even if the smell of concentrate helped the cows to find the right direction in the obstacle course (or barn) it would not help them to avoid obstacles. For this reason a bucket of concentrate is considered to be a good motivator. We cannot exclude that the obstacles also had a particular smell, but the obstacles were moved into the barn at least one day before the test started and fairly soon they should have taken the same smell as the environment.

It would have been interesting to monitor the HR during the entire tests. Then the fluctuations during the experiments could have been studied and together with behavioural observation a more detailed view on stress during different light intensities could have been seen. The design in this study did not allow us to see if the HR increased somewhere during the obstacle course or if it rose when they first detected the object in NOT. This could be a topic for further study.

This study was fairly small with only three cows in each group. Consequently the impact on the result due to each cow was rather large. For example the cow that tried to jump over the walls of the pen while vocalizing loudly is likely to have affected her measurements that day and it may not be representative for the population as a whole. On the other hand it is important to not exclude these individuals if it is not certain that only a very small part of the population reacts in such way.

The cows also differed in age, breed and time since pasture which, beside the personality of the cow, also can have an impact on the result. As mentioned before this was in some way counteracted by only comparing differentials. In this study the use of dried off cows was purely due to practical reasons. All cows were dried off and all but one was in the latter part of gestation, this is the features that set them apart from lactating cows. As a result they also are in different hormonal stages and lactating cows may have responded differently to the treatments. A cow in late gestation could be more drowsy than a lactating cow, both due to the fact that they carry around a quite big foetus and that the uterus presses onto the rumen, leaving less space for food hence lowering the cows feed intake. More energy is spent on gestation but none is spent on lactation, therefore the intake rather than the energy requirement is the limiting factor. Due to these reasons dried off cows may want to lay down more and are not as interested in spending time and energy on investigating objects. Several cows showed signs of late gestation when not handled, lying down on one side and breathing heavily.

To have light turned on during night time is a considerable cost for a production that today is on its margins. If the milk production is to continue in Sweden the viability has to increase and this starts with evaluating every cost. If light during the dark hours do not contribute in any way to the profitability the farmers should not be obligated to keep them on. If a threshold for the cows' ability to distinguish objects and the surroundings was found, that light intensity could be used as a recommendation during the night and be adjusted for the cows rather than to the workers (whose safety and ability to work of course have to be prioritised during working hours). There may also be animal welfare aspects to this issue. Which light and light intensity that is compatible with the physiological aspects of the cows and is most comfortable for the cows is not clear. Therefore it is important that further research in this area is made.

The results of this study indicate that cows are not stressed by low light intensities or darkness and based on this study alone it could be argued that no additional light is needed at all during night time. Similar to the results in this study, Pettersson and Wiktorsson (2004) could not see any preference at herd level for light or darkness. This stands in opposition to the hypothesis by Phillips et al. (2000) that cows would find darkness aversive. Because the cows in this study tended to show a stress response towards red light, it should be considered if red light should be used in the barns at all. In this study the activity was not significantly affected by light intensities but some indications towards more time spent lying down in 50 lux compared to the other light intensities were seen and should be more investigated in the future to ensure an environment for cows that facilitates the lying down behaviour.

Of course other considerations have to be made beside the cows' physiological reaction to low light intensities and darkness, such as behaviour and the incidence of injuries, before a final recommendation regarding night time light can be made. Further studies in this area are needed to find a threshold value for where cows start do distinguish their surroundings and hopefully a recommendation for light intensity and colours scheme for lights used during night time can be given in the future.

6. Conclusion

The different light intensities or the presence of red light did not affect the heart rate or blood pressure in dairy cows during their dry period. Additional red light showed signs of affecting the respiratory rate compared to only white light in dairy cows during their dry period. Also the respiratory rate was in some cases affected by the different light intensities while the activity was not.

These results indicate that cows are not stressed by low light intensities but can perceive red light and may be stressed by it. In order to ensure cows' need to rest and to cut costs 50 lux could be used during night time in stables. In extension this could lead to lower costs for the production and greater animal welfare for the cows.

Heart rate, mean arterial pressure and respiratory rate did not show any response to small changes in lighting. In further studies other parameters, such as different hormones, should be used.

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Appendix

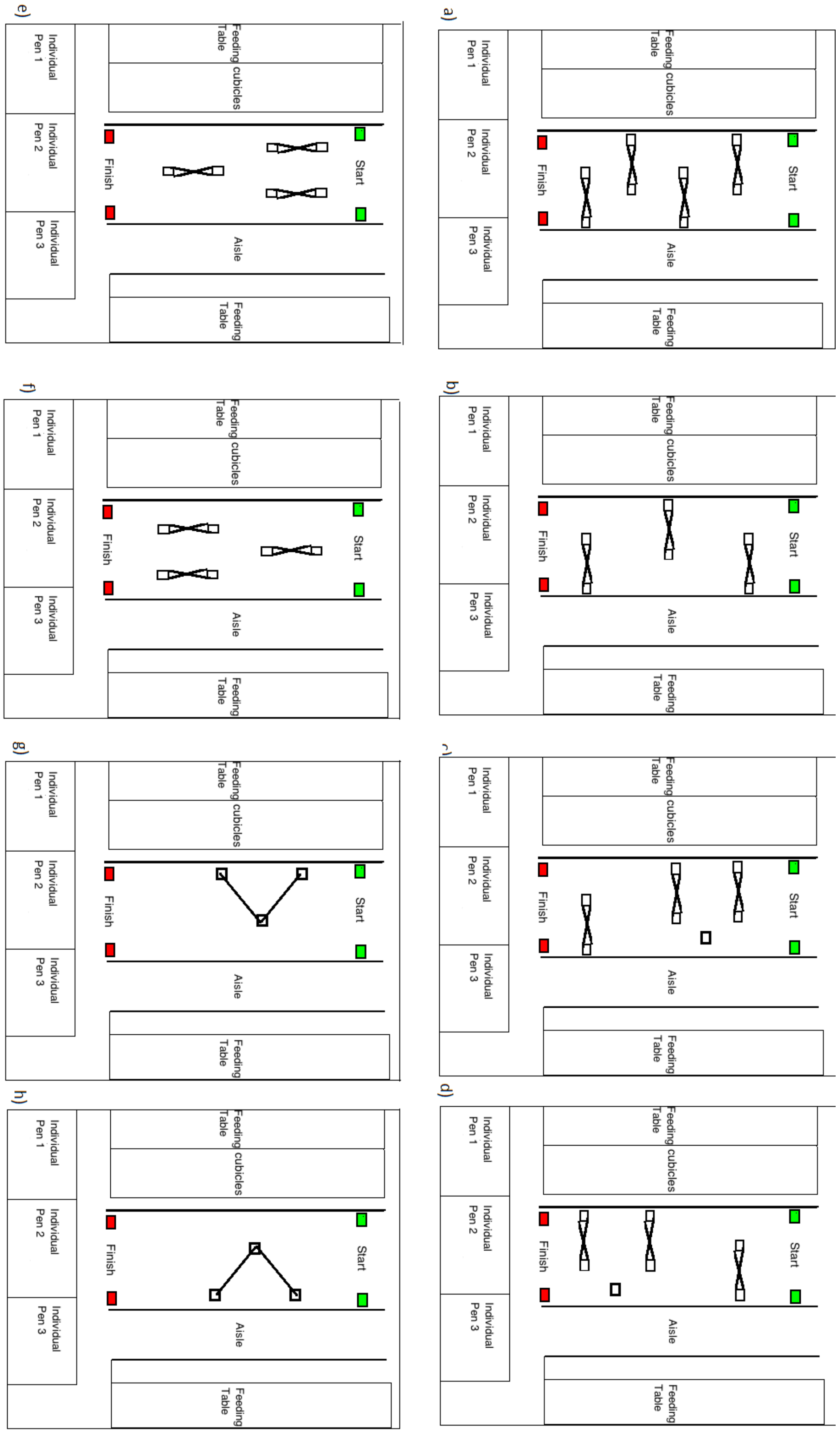
Appendix 1. Specifics of the cows used in the trial

Group	Cow id.	Breed	Age (years)	Lactatior nr	Days from drying of	Days to calving	Pen	Pasture[a]
1	1449	SRB	6	4	105	43	3	Yes
	1526	SRB	4	2	41	49	1	Yes
	1552	SRB	4	2	95	19	2	Yes
2	1518	SRB	5	3	49	44	2	Yes
	1556	SRB	4	2	89	48	1	Yes
	5357	SRB	5	3	113	48	3	Yes
3	1581	SRB	4	2	56	43	1	No
	1631	SRB	3	1	56	49	2	No
	6507	SH	3	1	56	54	3	No
4	1511	SRB	5	3	63	58	2	No
	1590[b]	SRB	3	2	63	[b]	3	No
	6522	SH	3	1	63	55	1	No
Average			4±1	2±1	70±24	46±10		

[a] Yes= directly from pasture to trial No= from indoors conditions to trial
[b] 1590 was not pregnant during the trial

Appendix 2. Unfamiliar obstacle courses

Unfamiliar obstacle courses



Appendix 3. Objects used in NOT and their dimensions

Object	Measurements
Red shopping bag	50*60*23cm
Blue IKEA bag	50*35*35cm
Pinwheel	Height 70cm, width of flower 25cm
Umbrella	Diameter 50cm, height 35cm
Pink box	35*30*20cm
Beach ball	Diameter 35cm
Swimming ring	Diameter 40cm, height 30cm

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