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Photo: Sabine Ferneborg

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Examensarbete / SLU, Institutionen för husdjurens utfodring och vård, **490**

Uppsala 2014

Degree project / Swedish University of Agricultural Sciences,
Department of Animal Nutrition and Management, **490**

Examensarbete, 30 hp
Masterarbete
Husdjursvetenskap
Degree project, 30 hp
Master Thesis
Animal Science

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Låga ljusintensiteters påverkan på mjölkkors beteende

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Omfattning:
Extent: 30 hp
Kurstitel:
Course title: Degree Project in Animal Science – E30
Kurskod:
Course code: EX0552
Program:
Programme: Master in Animal Science
Nivå:
Level: Advanced A2E
Utgivningsort:
Place of publication: Uppsala
Utgivningsår:
Year of publication: 2014
Serienamn, delnr: Examensarbete / Sveriges lantbruksuniversitet, Institutionen för husdjurens utfodring och vård, 490
Series name, part No:
On-line publicering: http://epsilon.slu.se
On-line published:
Nyckelord:
Key words: Light intensity, Dairy cow, Behavior, Vision, Color, Obstacle course, Novel object test

Abstract

The animal welfare act in Sweden states that dairy cows need to have at least a dim light present at night, but there are no recommendations for what intensity the light should have. It is unknown how cows perceive and react to low light intensities and earlier studies on this topic are limited. Red light as night light has also been suggested, based on the cows' inability to perceive red light. This have however been questioned.

The aim with this study was to investigate how four different light intensities affect dairy cows behavior. The aim was also to investigate how red light affects the cows' behavior.

In the study, twelve cows' behavior and locomotion pattern were observed at the different light intensities 0, 5, 20 and 50 lux in presence or absence of red light. The cows were asked to pass through an obstacle course and perform a novel object test (NOT) in the different treatments. The study design was following a change-over design with four groups of cows and four different light intensity treatments. The experiment prolonged for four weeks and each treatment period lasted one day.

Based on results in this study, it can be concluded that dairy cows can perceive light intensities down to 5 lux. It is further hypothesized that cows may be affected of a light intensity as low as 0.2 lux. This study also show that behavior is significantly affected by a light intensity since step frequency, number of stops, time taken for passing the obstacle course as well as step length and step rate were different at 0 lux compared to 5, 2 and 50 lux. In the NOT, time taken to the first interaction with an object was prolonged at 0 lux compared to the other light intensities.

The cows' behavior was not affected by the presence of red light in light intensities at 5, 20 and 50 lux. At 0 lux with presence of red light the cows had a higher frequency of steps, interacted less with the surrounding and knocked down fewer obstacles in the obstacle course compared to the other three tested light intensities. In the NOT, time taken for the cows to first interact with the objects was longer at 0 lux accompanied by red light compared to the other light intensities and compared to 0 lux in absence of red light. It is unclear if this is a result of a light intensity of 0.2 lux emitted by the red light or due to the precipitation of red light.

Sammanfattning

Enligt Sveriges djurskyddslag ska mjölkkor ha tillgång till dämpad belysning under natten, men det finns inga rekommendationer för hur stark belysningen bör vara. Hur kor uppfattar och reagerar på låga ljusintensiteter är ännu inte känt och det finns endast ett begränsat antal studier inom området. Nattbelysning som ger ifrån sig ett rött sken har introducerats i flera företag, med förklaringen att kor inte uppfattar det röda ljuset. Detta har dock ifrågasatts.

Syftet med denna studie var att undersöka hur fyra olika ljusintensiteter påverkar kors beteende. Syftet var även att undersöka om röd belysning påverkar deras beteende.

I studien observerades tolv kors beteenden i fyra olika ljusintensiteter, 0, 5, 20 och 50 lux i närvarande eller frånvarande av röd belysning. Korna manades igenom en hinderbana varefter ett novel object test utfördes i de olika behandlingarna. Utformningen av studien följde en change-over design av de fyra olika behandlingarna, där korna var indelade i fyra grupper om tre i varje. Experimentet pågick under fyra veckor, varav varje enskild behandling pågick under en dag.

Slutsatsen av studien är att kor kan uppfatta ljusintensiteter ner till 5 lux och troligtvis också ända ner till 0.2 lux. Deras beteende ändrades tydligt i 0 lux, jämfört med 5, 20 och 50 lux. Korna tog fler steg, stannade oftare, tog längre tid på sig i hinderbanan, minskade sin steglängd och steghastighet i hinderbanan i 0 lux jämfört med i de andra testade ljusintensiteterna. I novel object testet så tog det längre tid för dem att interagera med objekten i 0 lux jämfört med i de andra testade ljusintensiteterna.

Kornas beteende påverkades inte av den röda belysningen i de högre ljusintensiteterna 5, 20, och 50 lux. I röd belysning vid 0 lux tog korna fler antal steg, interagerade mindre med omgivningen, slog ner färre hinder i hinderbanan, medan det i NOT testet tog längre tid för dem att interagera med objekten jämfört med i 0 lux utan röd belysning. Om detta beror på att den röda belysningen ger ifrån sig en ljusintensitet på 0.2 lux eller att korna uppfattar den röda belysningen går inte att avgöra.

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1 Introduction

The sight is one of the most important senses for cows, and counts for approximately 50% of the total sensory information (Phillips, 2002). How cows perceive different light intensities is not yet known (Phillips et. al., 2000), but it is proven that cows sight differs from human sight (Phillips and Weiguo, 1990). This makes it difficult for humans to understand how cows visualize their surrounding. It is important to know how much the cows rely on their vision to be able to customize the cows' environment and the barn facilities. Visual ability can be observed and measured in different ways; through optometric measurements, measurements of the activity in the brain visual cortex or through observations of the animals' behavior when they are exposed to different stimuli (Phillips, 2002).

The animal welfare act in Sweden is highly elaborated and includes strict regulations concerning animal housing and management. The required light regime for dairy cows is that cows should have access to daylight during daytime and supplementary light should be provided during nighttime. It is also specified that dairy cows should have access to at least dim light during all 24 hours and that the light in the stable should be adapted so as to not causing any discomfort for the cows (SJVFS 2010:15). However, the animal welfare act in Sweden does not specify requirement and recommendations regarding the light intensity in the barn. This makes it possible for producers to evaluate the profitability in different light intensities by themselves. Today the automatic milking system, AMS, is commonly used in dairy farms in Sweden combined with a loosed-housing system. In these systems the cows can more or less choose when they want to be milked. The cows' movements in the stable, the cow traffic, in these systems can either be forced cow traffic when the movements are controlled by one-way gates, or free traffic with no gate system. The cows are supposed to find their way in the barn to the milking machine, the feeding stations and the resting area. Due to these expectations of the cows', their vision and mobility in these systems are important in order to get a high production (Phillips, 2002). Some producers using these systems prefer to use high light intensity for 24 hours. This is since they want to ensure that the cows find food and water supplies in the barn (Dahl, 2005). However, according to Dahl (2005) the cows have no problem finding their supplies in the barn in lower light intensities. Some producers also attempt to improve the production of milk by having a high light intensity for 24 hours (Gavan and Motorga, 2009; Miller et. al., 2000). It has been shown that if the cows are exposed for light 24 hours their daily rhythm are disturbed and their production does not improve (Hörndahl et. al., 2012). Light that is absorbed by the eyes is transported to the epiphysis in the brain, which manages the secretion of the hormone melatonin. The concentration of melatonin in the blood follows a daily cycle and during night, when the expose for light is low, the highest melatonin concentration in the blood occurs. The melatonin-levels regulate sleep and the secretion of hormones that regulates both reproduction and milk production (Hedlund et al., 1977). Baldwin and Start (1981) performed a preference test on individually housed calves to see how motivated they where to work for a light reward. The calves could control the lightening with their muzzles, through interrupt an infrared beam switch. The calves preferred to spend 67% of a 24 hours period in light, i.e. they preferred a day length of approximately 16 hours. Day lengths of 16 hours have also been seen to give the highest productivity compared to day length of 10 hours or 24 hours (Phillips, 2002). Different species have different light sensitivities and how sensitive the cows are to less lightening have not yet been studied (Hörndahl et. al., 2012).

To not disturb cows photoperiod and day length, at least six hours of darkness (<5 lux) per day is required, and the use of nightlight may interfere with this (Dahl, 2005). Some companies provide red night lighting, arguing that cows, in contrast to humans, do not perceive red light as light. The use of these lights makes it possible for the farmer to observe the animals without disturbing them (Dahl, 2005). Several researchers disagree with this and have shown that cows can perceive the color red and that their activity increases when they are exposed to red light (Dabrowska et. al., 1981; Riol et. al., 1989; Phillips and Lomas, 2001).

1.1 Aim and goal

The aim of this project was to investigate how four different light intensities affected the behavior in dairy cows. The aim was also to investigate how presence of red light affects their behavior. The goal is to achieve more knowledge about dairy cows' dark vision to be able to introduce appropriate and specified requirements for dairy cows.

1.2 Research questions

- Do cows' behavior differ in different light intensities?
- Are cows' interactions with unfamiliar objects affected by the light intensity?
- Do cows' react differently with or without presence of red light?

2 Literature background

2.1 Light intensity

The daily activity rhythms in mammals depend on several factors such as changes in light and dark, temperature, food and water supply. Among these factors the one that have the biggest influence on the daily activity rhythm are usually the light intensity (Piccione et al., 2011).

The light intensity is measured in lux and is the visible light emitted from a light source. The light intensity depends on the distance from the light source, the type of light source and the angle of the light. A light meter measures the light quantity at a specific place (Cheng et al., 2004). The distance from the light source is important when measuring the light intensity that the cows are exposed to. Previous studies of lighting in dairy barns have measured the light intensity on a height of 1.2-1.5 m, which is approximately the cow's eye height from the floor (Bal et al., 2008; Gavan and Motorga, 2009; Reksen et al., 1999; Stanisiewski et al., 1988).

The Technical Specification for dairy barns in Sweden (SIS-TS 37:2012) recommends a light intensity at 100-150 lux in a dairy barn, and a night light intensity at 5 lux. The recommended lowest light intensities differs in various countries, for example, in Germany and Great Britain the minimum recommendation during nighttime is at 20 lux, in France 30 lux, while in Switzerland the minimum recommendation is at 60 lux (Dannenmann et al., 1984; Phillips et al., 2000).

2.2 Cows vision

Cows are prey animals, making them very depended on their vision and they are easily motivated by fear. These animals have a panoramic vision of 360°, which enable them to distinguish moving objects behind them without turning their heads, while they have a poor depth vision. Cows usually avoid crossing shadows and changed texture or color on the floor, probably due to their lack of depth vision (Grandin, 1980).

The retinas of the cows' eyes are supplied with two types of receptors, rods and retinal cones. In dim light, the rods are active and enable the cows to see despite a low light intensity. In daylight the retinal cones are instead active and enable cows to see shapes and colors (Dannenmann et al., 1984; Sjaastad et al., 2010). The cows can perceive lower light intensities than humans, due to their higher amount of rods and their extra layer that reflects light on their eyes, called *tapetum lucidum* (Phillips et al., 2002; Sjaastad et al., 2010).

Cows have an ability to distinguish between basic shapes as triangles, squares and circles, as they are myotopic (Phillips, 2002). Cows vision differs from humans. Compared to humans, cows have a lower visual acuity, but their vertical acuity is better. Their lower visual acuity probably depends on their smaller amount of retinal cones in their eyes and their better vertical acuity base on their ovoid pupils. Cows have difficulties in focus the picture, since their pupils only can change shape vertical and they have a low ability to change shape of their lens. The picture is also more perceived in the outer part of the retina, which make the picture harder to focus (Phillips, 2002).

Cows are able to distinguish objects on a close distance in a light intensity of minimum 2 lux (Phillips et al., 2000). The visual capacity in 20 lux is better, which make it easier for the cows to perceive and detect a stimulus in this light intensity, but they still need to explore it on a closer distance for a final perception (Dannenmann et al., 1984).

2.3 Cows color perception

Light can appear in different wavelengths and together with the individual light sensitivity it appears in different colors. An objects color is determined by the objects' ability to absorb and reflect light. Most mammals can detect wavelengths around 400-700 nm. Mammals' eyes contain retinal cones, which make it possible for them to see colors. In low light intensities all vertebrates see in a black and white scale and colored objects is perceived as different shades of grey. Primates are called trichromats and have in contrast to the cows' three retinal cones. These cones make it possible for primates to perceive the colors red, green and blue (Sjaastad et al., 2010). The different cones are sensitive at different wavelengths. The color red reflect long wavelength, green reflect medium wavelength and blue reflect short wavelength (see fig.1) (Phillips and Lomas, 2001). Cows have only two types of retinal cones and are called dichromats (Sjaastad et al., 2010). It has been proven that the cows' two cones are most sensitive for maximum wavelengths of 455 nm and 554 nm (see fig. 1) (Jacob et al., 1997), and therefore it is believed that cows cannot distinguish medium and short wavelengths (green and blue) from long wavelengths (red) because these cones sensitivity are not in the long wavelength spectrum. Despite the cones sensitivities the maximum wavelength a cow can perceive are at 620 nm (Phillips and Lomas, 2001).

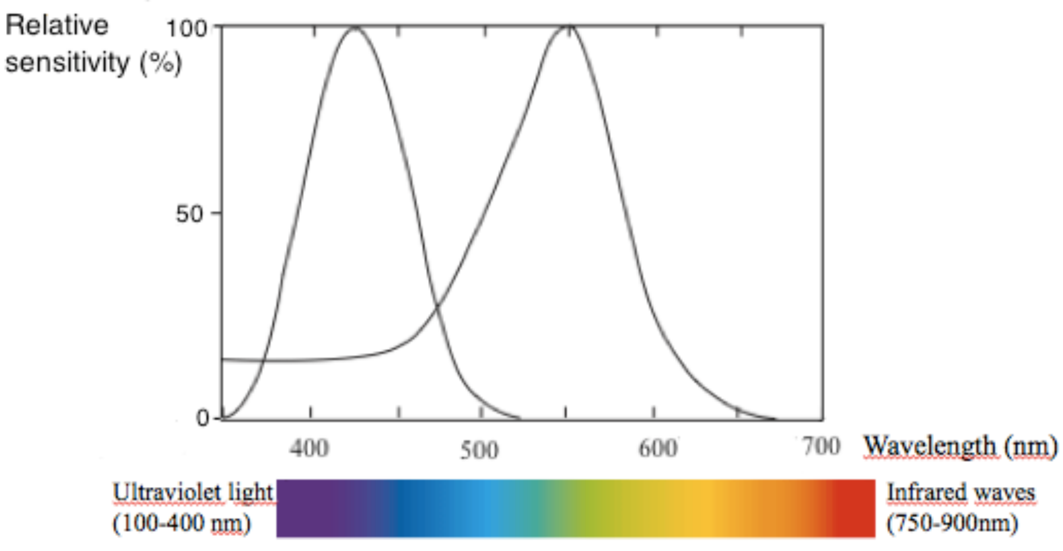

Fig. 1. Cows two cones sensitivity at different wavelengths and which wavelength that represents which color (Modified after Wallin, 2002 and Sjaastad et al., 2010)

However, it has been seen that cows can distinguish different colors, especially those in the long wavelengths spectrum (Dabrowska et al., 1981; Gilbert and Arave, 1986; Riol et al., 1989; Phillips and Lomas, 2001). Cows are prey animals and they were once depended on their vision in order to survive. Through their ability of perceiving the color red, they could distinguish blood and act fast when a herd member was attacked (Phillips, 2002). The researchers have instead questioned cows' perception of short wavelengths and their ability to differentiate short wavelengths (blue) from medium (green) (Dabrowska et al., 1981; Riol et al., 1989; Phillips and Lomas, 2001). However, it has been seen that cows have an ability to select between herbage of different shades of green, which reflects its nutrition content (Phillips, 2002).

Phillips and Lomas (2001) also observed that calves' behavior pattern changed when they were exposed for different colors of light. In long wavelength (red) they were more active and acted faster if they were exposed for a fearful stimulus than in short (blue) or medium (green) wavelengths.

2.4 Cows behavior and locomotion

A behavior can be defined as the animal's reaction to a specific stimulus. The animals' behavior pattern has evolved by the natural selection and origins in two evolutionary instincts, to stay alive and to reproduce. Behaviors can be studied in many different ways. Two examples are through direct observations or indirect observations. In the direct observation an observer watch the animals for a set time period and register its behavior. To not influence the animals' behavior while observing them the position of the observer is important. In the indirect observations video cameras are usually used to watch the animals (Blackshaw et al., 2003). Another possible way to study animals' behavior is using automatic methods, for example activity meters (McGowan et al., 2007). When studying animals behavior not only the frequency of the behavior is recorded, it also involves when, how, why and where the behaviors occurs (Blackshaw et al., 2003).

Cows' daily behaviors include standing, walking, lying, feeding, drinking, ruminating, and self-grooming (Mitlohner et al., 2001). In a study of lying and standing behavior, Stoye et al. (2012) observed a pattern in lying down and standing up, as the cows seemed to lie down and stand synchronized with each other, both for indoor conditions and at pasture. The cows lying and standing pattern were most synchronized during mornings and evenings. Cows on pasture lay down approximately half of the day to ruminate or sleep (Houpt, 1998). The environment and the cows' condition affect the time spent performing certain behaviors. For example, according to Albright et al. (1997), the time a lactating dairy cow spend lying down depended on type of housing, lying comfort, type of feed, pregnancy status and climate.

Locomotion can be defined as a voluntary movement that includes the entire body. Movement as walking, trotting or galloping is commonly occurring in cows. Other movements as jumping and swimming are also locomotion although performed less frequently. A faster gait generates a higher energy loss. Since the milk production is energy consuming for the cows, they prefer to walk to save energy. Their walk rate is about 0.6 – 1.0 m/s (Phillips, 2002).

Cattle are easily motivated to move to access different resources, such as feed or extra space. The cows' motivation increases with the duration, the availability and the severity of the resource. Several factors affect the locomotion in cattle, for example; the environment (such as climate, floor type, lightening, ventilation); the herd design (such as stock density, number of feeding place per animal, number of cubicles per animal); feed (the quality and quantity) and also genetic and social factors may have an impact on the locomotion (Phillips, 2002).

2.5 The behavior differences in light and dark

Cows' activity rhythms are assumed to be related to their constant discontinuation of predators. At pasture during daylight they usually expose themselves more to the predators performing activities including a lot of moving around, and feed and water intake while behaviors that can be performed lying down, as ruminating, are performed in the night when it is darker (Phillips and Arab, 1998; Dannenmann et al., 1984).

Phillips and Arab (1998) investigated if cattle prefer to perform certain behaviors in light or dark. Their experiment included four bullocks, which were housed in individual pens. They were able to control the light themselves through a light switch in the pen. The study showed that the bullocks preferred to feed in the light, although this correlation was not that strong. Feeding is a behavior that includes a lot of activity. The bullocks did not seem to prefer to ruminate more in either light or dark. They saw a tendency that the bullocks preferred to stand more in light than in the dark. However, the study also showed that the bullocks drive to perform behaviors uniformly as a group was stronger than performing a behavior in a specific light intensity (Phillips and Arab, 1998).

Dannenmann et al. (1984) conducted a similar experiment where they looked at differences in the behavior pattern in calves that were exposed to different light intensities. The calves showed a higher activity in light intensities at 100-130 lux than at 0-20 lux. In the lower light intensities the calves rested more, and if they interacted with any subject the interaction lasted longer than the interactions in the higher light intensity. The authors discussed whether the calves could not see the stimuli in the lower light intensities and therefore rested instead of take contact with it, or if the calves resting behavior was deranged due to the small difference between the light at day and night.

In a study by Phillips et al. (2000), dairy cows locomotion through different passageways was observed in six different light intensities between 0 and 250 lux. Number of steps and the time it took for each cow's hind limb to traverse through the marked passageways of 14 m was recorded. Step length and step rate was then calculated based on the results. The cows got 30 min to adapt to the set light intensity before each test. The results showed a change in the locomotion pattern in the dark (0-50 lux). The cows step length decreased, while their step rate increased in the lower light intensities at 0-50 lux. The cows took shorter but more rapid steps than in higher light intensities, which explain the increase of step rate in the lower light intensities. Phillips et al. (2000) discussed that the cows might feel unsafe in the darker area. Perhaps the cows rapidly wanted to return to their conspecifics on the other side of the passageway, they were probably also motivated by the food that was offered on the other side. In the same study it was also shown that the cows had a wider leg position when stepping through the passageways at lower light intensities. They describe this as an explanation for fear of slipping and a probably an attempt to improve their stability.

2.6 Cows avoid dark passageways

Studies have shown that dairy cows prefer to spend more time in light than in the dark (Baldwin and Start, 1981; Weiguo and Phillips, 1991). In a study by Phillips and Morris (2000), they presented a Y-formed maze with two different passageways for their seven experimental cows. One passageway had a light intensity at 22 lux, and the other one had a light intensity at 0.03 lux. Six of seven cows in the experiment choose to pass the passageway with the higher light intensity. The cows chose the brighter way even though reward was offered in the darker passageway. Phillips and Morris (2000) concluded that, if possible, cows avoid passageways of low light intensity. When the cows were herded one by one through a dark passageway they rushed through it. The authors suggested, like Phillips et al. (2000), that the cows preferred a higher light intensity because of their fear of slippery floors and invisible obstacles (Phillips and Morris, 2000).

2.7 Fear tests in cows

To study farm animals' fear, several different experimental designs exist. Fear tests commonly used in cows are Novel arena test and Novel object test (NOT), while other tests as forced and voluntary approach test are seldom used. In the Novel arena test variables as latency to enter and leave the arena, locomotion activity as walking and standing, exploration as sniffing on the environment, number of defecations and mictions, vocalizations and escape attempts are recorded. In these experiments the animals are usually tested individually and each trial persists 1-30 min. This test is commonly combined with a separate Novel object test. The repeatability of this test has been questioned, as the wanted unfamiliarity in the environment becomes familiar when the animal has been tested once. Locomotion, vocalization, time spend standing still and exploring are however the most repeatable variables (Forkman et al., 2007).

Novel object test is the second most common fear test (Forkman et al., 2007). It usually persists 1-15 min and is performed individually in a pen. The test is usually visual; an unfamiliar object is put on the floor in the pen by a human or dropped on the floor from the ceiling. The commonly recorded variables are; the interest towards the object (measured through the time to first interaction), distance from the object, frequency of interactions, the time of each interaction, exploration as licking or smelling, body posture and vocalization. If a novel object test are repeated eight times in one week a general decrease in exploring behavior has been recorded in earlier studies (Forkman et al.,2007).

The forced and voluntary approach tests are fear tests that investigate the cows' reactions to humans. In the forced approach test, the human approaching the cows, without the cows' consent, while in the voluntary approach test, the cows' contact with the human is voluntary. These tests are usually combined with other fear tests and usually comparing with or without presence of conspecifics (Forkman et al., 2007).

2.8 Activity meters to record behaviors

Activity meters can be used for many species to investigate different behavior patterns and movements. They measure two different kinds of forces, static (as gravity) and dynamic (from movements or vibrations) (Miedema, 2009).

Activity meters measure the acceleration, the time rate of change of distance, in meters per second squared (m/s^2) (Miedema, 2009). The activity meter is depending on the gravity and if the activity meter is placed vertical (when the cow is standing) it gives a value of 100% and if it is placed horizontal (when the cow is lying), it would generate a value of 0%.

3 Materials and Methods

The experiment was performed at the Swedish Livestock Research Centre, Lövsta. The trial followed a change-over design with four groups of cows and four different light intensity treatments. The four different treatments at 0, 5, 20 and 50 lux were investigated in two different ways in the study, through an obstacle course and a Novel object test (NOT). The objectives for both parts were the same, to study if the cows' behavior alters with lower light intensities and to study if it differs any in presence of red light.

3.1 Animals and housing

The experiment was conducted during four consecutive weeks, 2013-10-08 – 2013-11-01, with three cows during each week, totally twelve cows. The Uppsala animal ethics committee had approved all animal handling included in the trial. The number of cows was selected with consideration of the study design of the division of four groups of cows and the length of the trial. The division of groups was made to match the number of individual pens in the stable.

The cows included in the experiment were of Swedish red (n=10) and Holstein (n=2) breed. The cows were selected with consideration of criteria as: stage of lactation (dry), parity and good hoof and leg health status. The selected cows' properties are listed in table 1.

Table 1. The properties of the selected cows in the experiment, which group they belonged to in the experiment, their ID number, their breed, their date of birth, their lactation number, their number of days since dry of and their number of days to calving.

Group	Cow ID	Breed	Birth	Lactation number	Days since dry off	Days to calving
1	1526	SRB	2009-01-05	2	40	50
1	1552	SRB	2009-07-07	2	93	20
1	1449	SRB	2007-10-04	4	104	44
2	1556	SRB	2009-07-20	2	87	49
2	1518	SRB	2008-11-14	3	47	45
2	5357	SRB	2008-10-12	3	111	49
3	1581	SRB	2009-11-25	2	54	44
3	1631	SRB	2010-07-27	1	54	51
3	6507	Holstein	2010-02-17	1	54	55
4	6522	Holstein	2010-09-08	1	61	56
4	1511	SRB	2008-10-11	3	61	59
4	1590	SRB	2010-01-01	2	61	-[1]

[1]Cow nr 1590 were not pregnant during the experiment

One day before the experiment started, the cows were moved to the stable for acclimatization. The cows had free access to forage during the days of trial, the first feeding occasion each day was at 7.00 am and the last was at approximately 7.00 pm. The water supply was *ad libitum* through automatic water cups in the pens. The pens were cleaned at least twice daily, 7.30 am and 6.30 pm. If necessary, they were cleaned more often. The bedding material in the pens consisted of wood shavings.

In the stable there were three individual pens equipped with headlocks, a narrow herding aisle, a wide aisle and one row with ten tie-stalls and a feeding table (see illustration and measurements in fig. 2). The stable had twelve fixed fluorescent lamps.

The flooring in the wide aisle consisted of a field with concrete floor in the middle of the aisle, approx. 0.7 m width, and rubber mats on the rest of the floor.

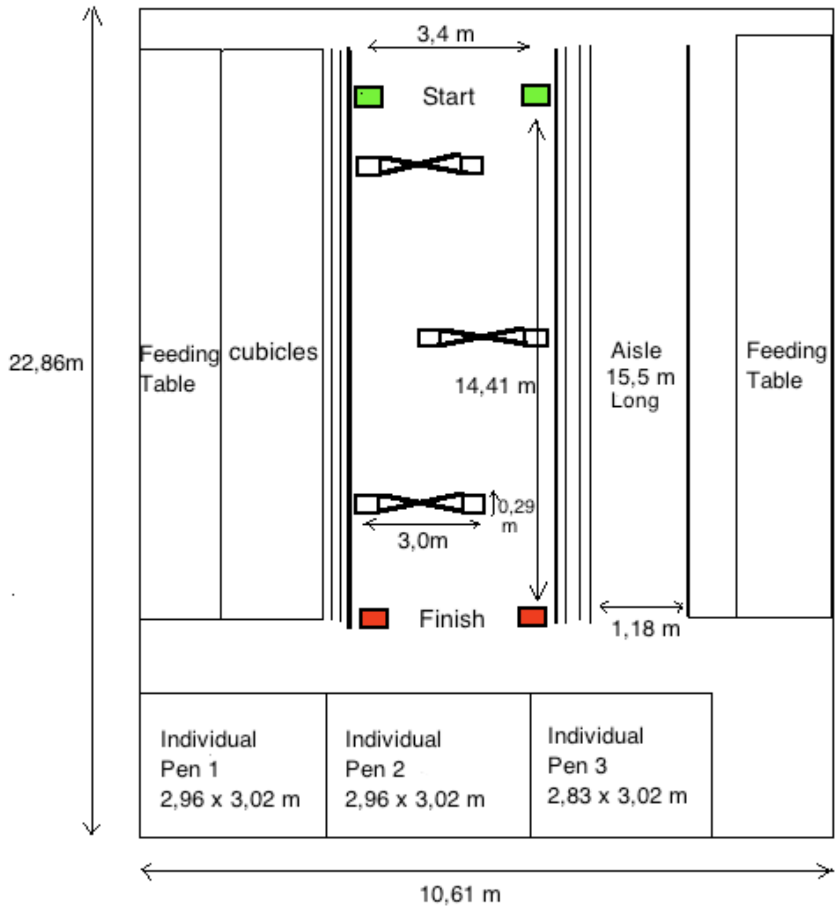

Figure 2. Illustration of the experimental stable and the familiar obstacle course design (not drawn in scale). The arrows show the measurements between two interiors. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The colored squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrate the two slatted floors.

3.2 Experimental design

The effect of four different light intensity treatments was explored, each treatment period lasted one day. To exclude the influence of group and previous exercised light levels, the experiment was designed as a change-over study. This design was used to reduce the error rate through minimize the effects of the individual cows and external factors. The light intensity in the obstacle course was not even distributed, so the light intensity were measured in six different places with a distance of approximately 2.9 m in the center of the aisle and an average was counted (see light intensities in table 2).

Table 2. The average light intensities ± standard error used in the obstacle course

Group	**50 Lux**	**20 Lux**	**5 Lux**	**0 Lux**
1	49±30 lux	20±8 lux	5±1 lux	0±0 lux
2	50±25 lux	19±6 lux	5±1 lux	0±0 lux
3	50 ±26 lux	20±7 lux	5±1 lux	0±0 lux
4	51±27 lux	21±6 lux	5±1 lux	0±0 lux
Average of the light intensities ± SE	50±2 lux	20±1 lux	5±1 lux	0±0 lux

The light intensity was measured with a light meter (Standard ST-1300) at a height of 0.8 m. The light intensities were achieved through present of light strands, mounted on gates that fenced the obstacle course. The fluorescent lights in the ceiling were covered with black plastic bags to different extent depending on the light intensity. To achieve the higher intensities at 50 and 20 lux a construction lamp was placed outside the obstacle course directed towards the course on a distance of 2.2 m from the middle of the obstacles course. At the light intensity 0 lux all light sources were completely covered. The whole study was video recorded and five infrared lamps were mounted on the walls, which made it possible to observe the cows through the cameras even in 0 lux.

3.2.1 Activity meters

Activity sensors exist in different types and in the present study IceTags (IceTag, Icerobotics Inc., Edinburgh, UK) have been used. This activity sensor can be used in research to measure and observe cows' behavior pattern. The IceTag are mounted on one hind limb just above the hoof, where it records the time the cows spend standing and lying (McGowan et al., 2007).

The first morning of trial, starting week 2, each cow was equipped with activity meters (IceTag, Icerobotics Inc., Edinburgh, UK) on the rear left limb registering the time of standing and lying. The cows got approximately 45-60 min to adapt to the activity meters. The activity meters were attached to the cows for the four days of trial.

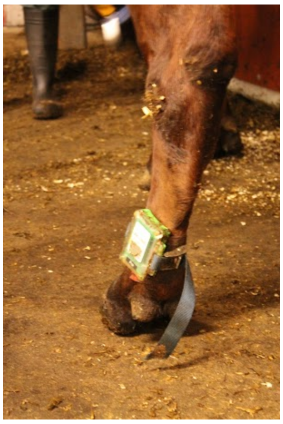

Fig. 3 An activity meter attached to the cows rear left limb.
Photo: Sabine Ferneborg

3.2.2 Herding aisle

Before the cows entered the obstacle course they were first herded through the narrow herding aisle of 15.5 m in the four different treatments. Their frequency of steps was registered and their step length was later calculated with the information. At the end of the herding aisle the cows pulse, blood pressure and respiratory rate was measured and registered for another experiment.

3.2.3 Obstacle course

The experiment was divided in two parts, consisting of an obstacle course and a Novel object test. Each day of the trial period looked the same according to a predetermined day-schedule, always started with the obstacle course and ending with the novel object test.

The cows were always first presented to an obstacle course in full light (221 lux). The cows were herded to a specific starting point. When the cow's hind legs passed the starting point the time (seconds) to the hind legs passed the marked finish line was recorded along with a number of other behaviors shown in table 3. The cows' behaviors were observed and registered as the cow went through the obstacle course. The cows passed the obstacle course individually, starting with the cow in pen 1 and ending with the cow in pen 3. Which cow in which pen was selected randomized. The cows were enticed to pass through the maze by concentrate and herded when needed.

Table 3. A ethogram of the parameters that were observed and recorded in the different treatments in the obstacle course and Novel object test included in the experiment

Observation	**Definition**	**Unit**
Step	Left hind leg was lifted up in the air and put down on the ground again.	No.
Interaction	The cow, with any part of the body, sniffing, licking or deliberately touching an object or obstacle.	No.
Interaction with surroundings	The cow sniffing, licking or deliberately touching, with its nose or another body part, a surrounding gate or the floor.	No.
Knock-down	The cow touches an obstacle and the obstacle fall down.	No.
Jump over obstacles	The cow jump/step over the obstacles without knocking it down.	No.
Stand still	The cow stand with at least 3 hoofs on the ground for at least 5 seconds.	No.
Slipping	The cow not deliberately does a sliding movement with a leg along the floor.	No.
Vocalization	A sound created by the cows' vocal cord, loudly of at least one second.	No.
Defecation	The cow defecated.	No.

When the correct daily light intensity had been set, the cow was allowed to adapt to it for 30 min. Then they individually passed the same obstacle course two more times with or without presence of red light in different order. The order of the presence of red light was randomized to minimize the effect of the number of day and trial. The red light emitted a light intensity of 0.2 lux. Same behaviors were observed like in full light (table 3).

The obstacles in the obstacle course were then moved and the places of them were changed, and the new obstacle course was called the unfamiliar obstacle course. The cows passed the unfamiliar obstacle courses two times, in a randomized order of presence and absence of red light. Between the two runs the places of the obstacles in the course were changed, to keep it unfamiliar. The places of the obstacles in the unfamiliar course were predetermined and used in the order b to i (illustrations in appendix 1). The same behaviors as in full light and in the familiar obstacle course were observed and registered.

The obstacles in the obstacles course consisted of white colored bars on plastic blocks. Each cow went through the obstacles course totally five times each day, the first time in full light, as a test drive, two times in a course with obstacles in the same places as in full light and two times in two different courses with obstacles in new places.

3.2.4 Novel object test

The light intensity was measured in the center of each pen at a height of 0.8 m. Since the light sources were not evenly distributed in the stable, the measured light intensities in the pens differ from each other and from the obstacle course (table 4). The cows got 30 min to adapt to the light intensity before the Novel object test started.

Table 4. The average light intensities ± standard error used in the Novel object test

Group	50 lux	20 lux	5 lux	0 lux
1	45 ± 2	20 ± 1	5 ± 0	0 ± 0
2	48 ± 2	20 ± 1	5 ± 0	0 ± 0
3	50 ± 0	20 ± 0	5 ± 0	0 ± 0
4	50 ± 1	21 ± 1	5 ± 0	0 ± 0
Average of the light intensities ± SE	48 ± 1	20 ± 1	5 ± 0	0 ± 0

The cows where in their home pens and a, for the cows, unfamiliar object was put by hand in the rear right corner of the pen. The same corner was used for all cows in every trial to make the trials as uniform and as easy to compare as possible. Both the order of the cows and the objects was randomized. When the object was placed in the pen the cow was not allowed to eat or lay down. If they lay down they were forced up and if they had their heads out through the headlocks, their heads were forced inside. The time measure and behavioral observations started as the object was placed in the pen. The time it took for each cow to first interact with the unfamiliar object, measured in seconds from the second the object was placed in the pen to when the cow made any contact with it, sniffing, licking or touching, were recorded. The length of each interaction measured in seconds and the number of interactions with the objects was also measured, counted and registered (table 3).

Each novel object test persisted for 15 min and each cow was subjected to two trials, with or without presence of red light at a specific light intensity. The presence of red light was also in this part of the experiment randomized.

Eight different objects were used in the novel object test to avoid presenting the same object several times for one cow. Objects that were used in this experiment were an umbrella, a pink upside down turned box, a beach ball, a swimming ring, a pinwheel, a red plastic bag, a blue plastic bag and an orange cone (pictures and measurements in appendix 2).

3.3 Data handling

The data from the observations was statistically handled using the statistical model "the mixed procedure" (proc mixed) in the software Statistical Analysis System (SAS, Cary USA, version 9.3). This model was selected due to its ability to conclude fixed, repeated and random effects. The observations of vocalization and slipping was excluded in the statistical analyze, due to their low frequency.

Repeated subject effect in the model was cow. Fixed effects in the model used for the obstacle course variables were light intensity and light color (presence of red light or not), while random effects were number of days in trial, number of obstacle course and the light intensity x light color interaction as following;

$$Model\ 1: Y_{ijklm} = \mu + LI_i + LC_j + D_l + C_m + (LI_i \times LC_j) + e_{ijklm},$$

Where Y_{ijklm} is the behavior effect of the treatment, μ the total mean, LI_i, the light intensity i=0, 5, 20, 50; LC_j the light color effect j=red light, no red light; D_l is the effect of the number of days in trial l=1, ..,4; C_m is the number of obstacle course $m=1, ..,9$, LI_i x LC_j is the effect of light intensity x light color interaction, and e_{ijklm} the residual error.

Repeated subject, fixed and random effects in the model of the NOT variables were the same as the obstacle course variables, except that the number of obstacle course were replaced with the number of object as following;

$$Model\ 2: Y_{ijklm} = \mu + LI_i + LC_j + O_l + D_l + (LI_i \times LC_j) + e_{ijklm},$$

where O_l, is the number of object l=1, .., 8. The rest of the variables as model 1.

The data from the activity meters, the frequency of standing and lying, was also statistically analyzed through SAS, using the model proc mixed. Repeated subject effect in the model was cow. Fixed effects in the model used on the activity meters variables were light intensity;

$$Model\ 3: Y_{ij} = \mu + LI_i + e_{ij},$$

where, Y_{ij} is the time spent standing or lying in the treatment. The rest of the variables were as in model 1.

The data is presented with the average ± SE of every cow's observations in each light intensity and in presence of red light or not.

4 Results

4.1 Obstacle course variables

The number of steps in the obstacle course was significantly higher in 0 lux (16 steps) compared to 5, 20 and 50 lux (14 steps) ($p<0.001$) (See fig. 4). In absence of red light the cows had a significant higher number of steps through the obstacle course (15 steps) than in presence of red light (14 steps) ($p<0.01$) (see appendix 3).

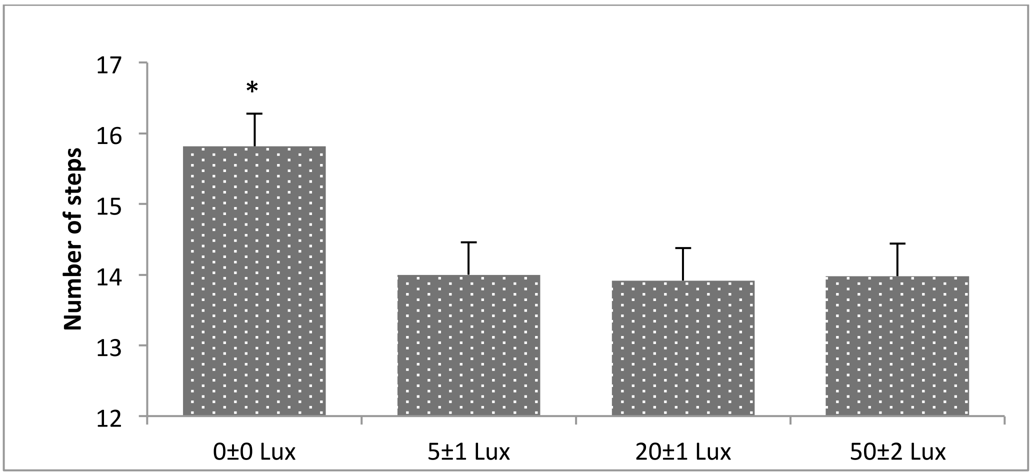

Fig 4. The cows' number of steps ± SE through the obstacle course at the four different light intensities ± SE included in the experiment
*p<0.001

The step length was shorter in 0 lux (1.16m) compared to 20 lux (1.23m) ($p<0.05$) (See fig. 5). The number of steps and the step length through the aisle did not differ in presence of red light (see appendix 3).

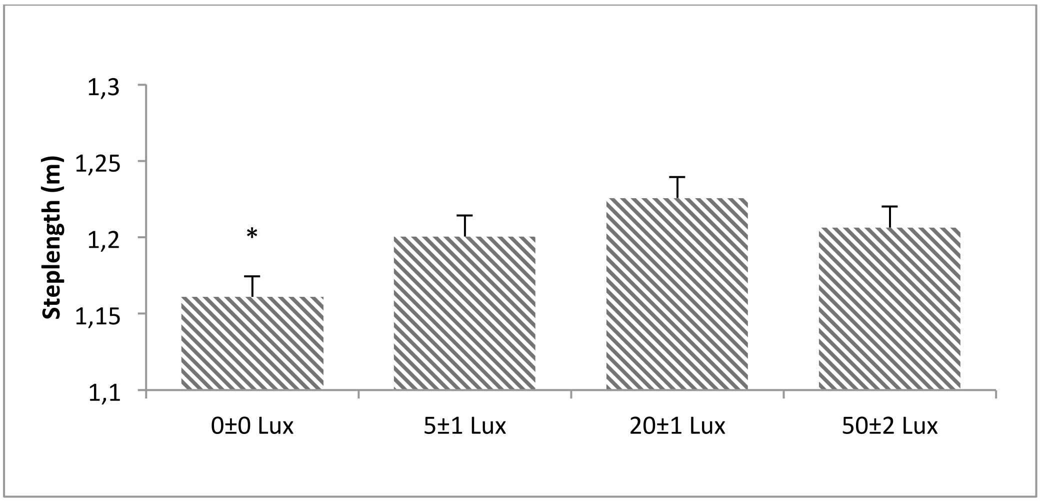

Fig 5. The cows' step length ± SE through the obstacle course at the four different light intensities ± SE included in the experiment
*p<0.05

The cows spend longer time in the obstacle course at the light intensity 0 lux (87 sec) compared to both 20 and 50 lux (51 and 58 sec respectively) ($p<0.005$) (see fig. 6).

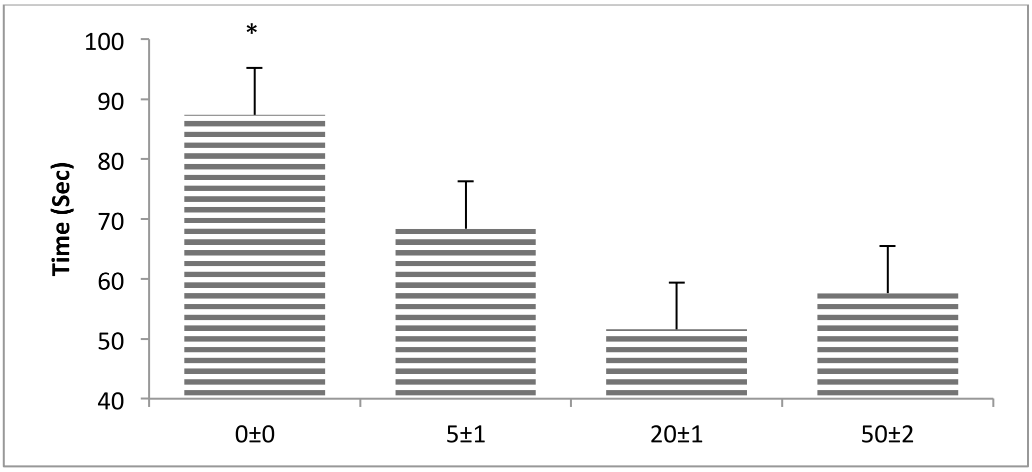

Fig 6. Time spend in the obstacle courses ±SE by the cows at the four different light intensities± SE included in the experiment
*p<0.005

The cows also stopped more frequently in 5 lux than in 20 lux (p<0.05). The presence of red light did not affect, neither the time spent nor the frequency of stops in the obstacle course.

In 20 lux the cows showed a higher step rate (0.37 steps/sec) compared to 0, 5 and 50 lux (0.30, 0.27 and 0.30 steps/sec respectively)(p<0.05) (appendix 3). The presence of red light did not affect the step rate through the obstacle course.

4.1.1 Interactions in the obstacle course

The cows interacted significantly more with the obstacles in 0 lux (2 interactions) compared to in 20 and 50 lux (1 interactions respectively) (p<0.01) (See fig. 7).

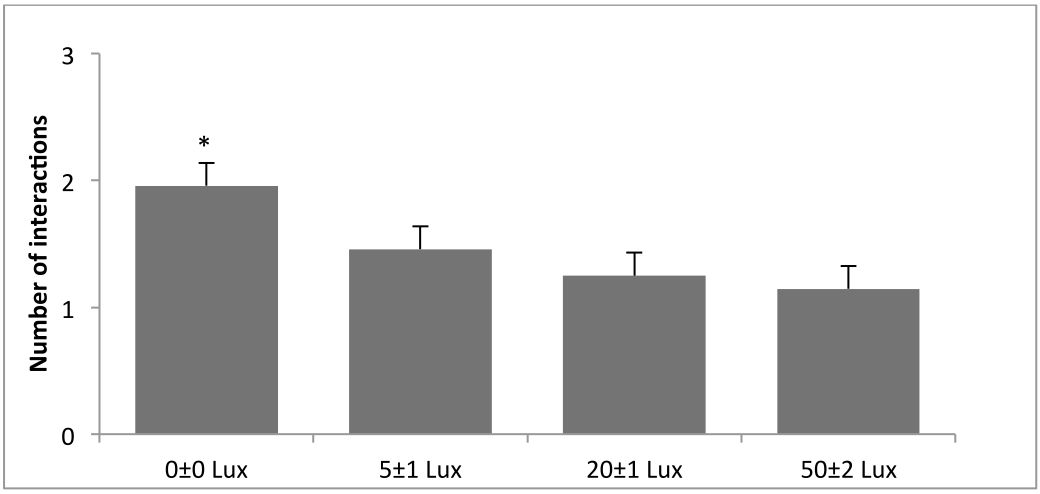

Fig 7. Number of interactions with the obstacles in the obstacle courses ±SE at the four different light intensities± SE included in the experiment
*p<0.01

In 0 lux the cows also defecated less than in 50 lux (p<0.05) (appendix 3).There were no significant differences in the number of jumps over obstacles, knock down of obstacles or interactions with surroundings between the treatments (appendix 3).

The cows did not interacted any different towards the obstacles, jumped over the obstacles more or less times, defecated more or less or stopped more or less frequent in the obstacle course in presence of red light compared to absence of red light. However, in red light the cows knocked down more obstacles ($p<0.05$) and interacted more with the surrounding ($p<0.05$) than in absence of red light (appendix 3).

4.1.2 Effect of the variables number of day and obstacle course

The cows took significantly longer steps day 1 compared to day 4 ($p<0.005$), they also interacted more with the surroundings day 1 than day 3 and 4 ($p<0.05$).

In the obstacle courses b, c, d and e, the cows significantly knocked down more obstacles ($p<0.05$) compared to the other courses.

4.1.3 Light intensity and light color interaction

The cows had a higher frequency of steps and it took longer time for them to pass the obstacle courses in 0 lux compared to 5 lux, 20 lux and 50 lux in absence of red light and 0 lux in presence of red light ($p<0.05$).

4.2 Novel object test

Latency to the first interaction in the NOT was significantly longer in 0 lux (512 sec) compared to both 5 and 50 lux (285 and 291 sec respectively) ($p<0.05$) (fig. 8).

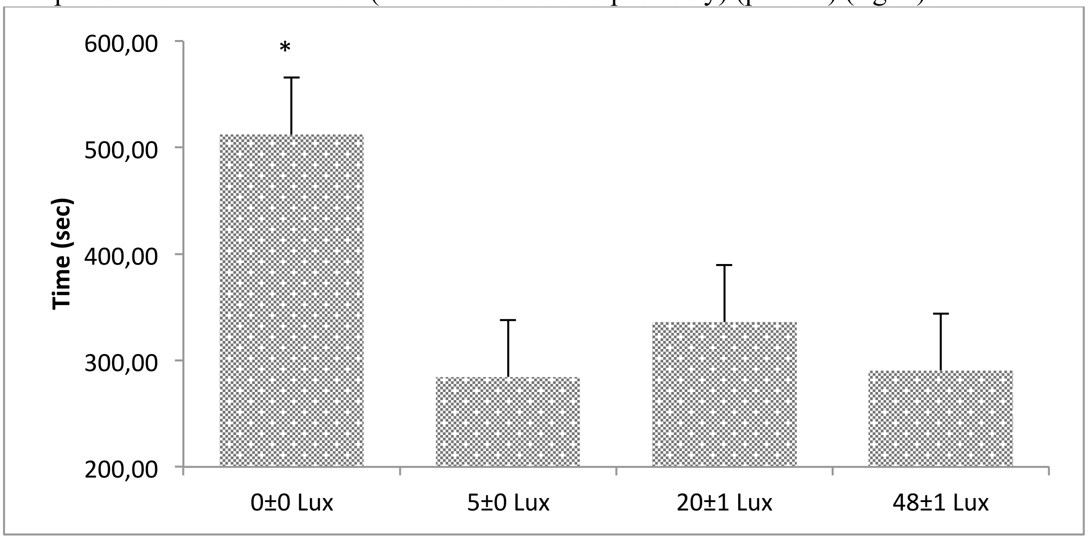

Fig. 8. The average time it took for the cows to first interact with the unfamiliar objects at the four different light intensities
*p<0.05

The cows interacted more with the objects in 50 lux compared to 20 lux ($p<0.05$) (appendix 4). There was no significant difference in either the time to first interaction and number of interactions with the unfamiliar objects in presence of red light.

The average lengths of the interactions with the objects did not differ significant between the different light intensities or between presence of red light or not (appendix 4).

4.2.1 The effect of day and object

Day 1 and 2 differed significantly from day 4 in number of interactions. The cows interacted fewer times with the unfamiliar objects day 4 than day 1 and 2 ($p<0.05$).

The type of object also seemed to have an impact on the cows' behavior in the different treatments. The cows interacted fewer times with object g (the swimming ring), compared to all other objects ($p<0.01$). In presence of red light the cows interacted fewer times ($p<0.05$)

with object b (the orange cone) compared to the other objects but the interaction lasted longer ($p<0.05$).

4.2.2 Light intensity and light color interaction

There was an influence between light intensity and presence of red light or not. It took significant longer time for the cows to first interact with the unfamiliar objects in 0 lux in presence of red light and 5 lux, 20 lux and 50 lux in absence of red light ($p<0.01$).

4.3 The activity meter data

There was no significant difference in time spend lying and standing in the different treatments, but a tendency that the cows spend more time standing in 20 lux compared to 50 lux ($p=0.07$) could be seen (table 5).

Table 5. Percentage time spend standing and lying in the different treatments.

Treatment (Lux)	Time spent standing	Time spent lying
0±0	46.6%	53.4%
5±1	45.1%	54.9%
20±1	49.3%[1]	50.7%[1]
50±2	40.4%	59.6%

[1]p=0.07

5 Discussion

5.1 Obstacle course

The study showed a distinct alteration in the observed behaviors at the light intensity 0 lux compared to the intensities 5, 20 and 50 lux.

At 0 lux the cows increased their step frequency and their step length was shorter compared to the higher light intensities (5, 20 and 50 lux). These results correspond to other researchers findings. Phillips et al. (2000) showed a higher frequency of the cows' steps and a shortening of their step length in low light intensities (0, 0.7, 4.3, 32 lux). However, their results did not completely correspond to the result in the present study. Phillips et al. (2000) also saw a higher step rate at the low light intensities (0, 0.7, 4.3, 32 lux) than at the high light intensities (119, 250 lux), whereas in the present study the result indicates a lower step rate at the low light intensities (0 and 5 lux). In the present study the cows stopped more frequently in the obstacle course at the lowest light intensity (0 lux) than in the higher light intensities (20 and 50 lux) and it took longer time for them to pass through the course compared to the higher light intensities. As mentioned earlier, the cows had a higher frequency of steps through the obstacle course, shorter step length and stopped more frequently at 0 lux. These properties affected the cows' time through the obstacle course, which in turn affected the cows' step rate. That the cows stopped more often, took shorter and more frequent steps in the lower light intensity (0 lux) may be due to their impaired vision and fear of slipping like other researchers earlier have suggested (Phillips et al. 2000; Phillips and Morris, 2000).

The obstacles in the obstacles course consisted of white-colored bars on plastic blocks. The color of the obstacles was chosen with consideration of how cows see and how different colors might influence them. For example, red colors have been shown to induce stress in cows and green colors induce calmness, while blue colors are hard for cows to see sharply (Phillips and Lomas, 2001). As little influence of the colors as possible was wanted. It was not of preference that the colors of the obstacles affected the cows more than the light intensities. It was also important that all the obstacles were in the same color so that the cows did not react differently on different obstacles.

Dannenmann et al. (1984) showed that calves interacted more with objects in high light intensities (100-130 lux) than in low light intensities (0, 5 and 20 lux). In the obstacle course in the present study the cows interacted more with the obstacles in 0 lux than in the higher light intensities of 20 and 50 lux, which do not correspond to Dannenmann et al. (1984) results. The increase of interaction at the lower intensity maybe due to cows' inability to see the obstacles and that they therefore interacted with them to localize them, or that the cows interacted with them unintended. At the higher light intensities (≥20 lux) the cows maybe had a better vision and could observe all obstacles and easily find a way to walk around them. During the study it was observed that the cows seemed more playful towards the obstacles in the higher light intensities, while in the lower ones the cows were more hesitant and cautious towards them. However when comparing this study with Dannemann et al. (1984) study it is important to add that in this study the high light intensities were at 20 and 50 lux, while Dannenmann et al. (1984) had a high light intensity level at 100 and 130 lux.

Defecation can be an indicator of stress (Phillips, 2002). However, in the present study this was probably not the case. Between the trials the cows often lay down and when they were asked to stand up for trial they usually defecated. The cows defecated significantly more in higher light intensities (50 lux) than in lower (0 lux), but only based on these results a conclusion whether the cows were stressed or not during the trial in the different treatments cannot be drawn.

The number of days that the cows were participating in the experiment seamed to have an influence on the cows' step length and their interactions with the surroundings of the obstacle course in the different treatments. The cows took longer steps and interacted more with the surroundings the first day of the week compared to the last ones. This might be because of the adaption to the environment and the knowledge of the procedure. If the cows got one day to perform the experiment without recording, they would know what was expected of them and the environment would be familiar day one as well. The first part of the experiment performed in this study had some similarities with the Novel arena test, and Forkman et al. (2007) have questioned the repeatability of this test, because the wanted unfamiliarity in the environment. They however claimed that the most repeatable variables were locomotion, vocalization, times spend standing still and exploring.

The number of obstacle course also seemed to have an influence on the cows in the different treatments. The cows always went through a course (a) with obstacles at familiar places first, two times, and then they went through two courses with different design and obstacles in unfamiliar places (b-i) (see pictures in appendix 1). In the unfamiliar courses b, c, d and e, the cows knocked down more obstacles ($p<0.05$) compared to the other courses. This is probably an indication of these designs of obstacle courses being more difficult for the cows to pass through. Some of these courses included more obstacles and in all four of them the obstacles were placed more horizontal compared to the other four unfamiliar courses (f, g, h, i). In the other four courses the obstacles were placed more or less vertical and probably did not stand in the cows' way as much as the courses b to e. The sight is the most important sense for the cows (Phillips, 2002), but they are also known to be creatures of habit, and maybe they only went through the unfamiliar obstacle courses by habit of the familiar one and knocked down the obstacles that stood in their way. It could also have an explanation in the cows' possible inability to see any other way and needed to step over the obstacles.

As to the effect of presence of red light or not, the cows had a lower frequency of steps, interacted more with the surroundings and knocked down more obstacles in presence of red light in all the tested light intensities. This could be explained by the red light emitting some light (0.2 lux). Looking at the effect of the different light intensities in presence of red light or not, the cows spent longer time in the obstacle course and had a higher frequency of steps through the course at 0 lux in absence of red light compared to 0 lux in presence of red light and 5, 20 and 50 lux regardless of red light presence or not. This was probably due to the cows' low ability to see at 0 lux in absence of red light. This result shows that cows probably can perceive objects or at least contours in either presence of red light or due to the presence of a light intensity at 0.2 lux that the red light emitted, only based on this results, a conclusion of either of them cannot be stated.

5.2 Novel object test

In the Novel object test, it took longer time for the cows to first interact with the objects and the cows interacted fewer times with them in the lowest light intensity (0, 5 lux) than in the highest one (50 lux). An explanation for this could be that the objects maybe perceived differently for the cows in the lowest intensities, so they were more afraid of them and did not want to make contact with them. Another explanation could be that cows might only be able to distinguish objects in a light intensity of minimum 2 lux (Phillips et al., 2000). Maybe the cows did not perceive the objects in the lowest intensities (0, 5 lux). Cows have a higher visual capacity in 20 lux compared to 2 lux, and they usually interact longer with objects to characterize and determine them in 20 lux (Dannenmann et al., 1984), which can explain the higher frequency of interaction with the objects in the higher light intensity (50 lux).

There were no significant differences in the average length of interactions with the different objects between the light intensities. This can indicate that the objects were equally interesting for the cows, which was good. It was important that the objects were as similar as possible to be able to compare the different treatments.

Looking at the objects' effect on the cows' behaviors in the different treatments it appeared as the cows interacted fewer times with the object g (the swimming ring), compared to all other objects. This object was maybe more frightening for the cows than the other ones, or vice versa, too outstanding. So, we can conclude that the object g was perceived as different to the other objects by the cows. But the other objects were probably perceived as similar.

Number of day in trial had an influence on the behavior of the cows both in the obstacle course and in NOT. In the NOT, the cows interacted fewer times with the unfamiliar objects day 4 than day 1 and 2 ($p<0.05$). This result corresponds to other researchers result. A general decrease in the exploring behavior has been seen combined with a increase in number of days in trial (Forkman et al., 2007). The cows probably have knowledge of the procedure the last day and know that none of the objects are of interest for them.

At 0 lux in absence of red light it took longer time for the cows to first interact with the objects compared to 0 lux with presence of red light, and 5, 20 and 50 lux in presence of red light. These results correspond to the results presented for the obstacle course and are probably due to the same thing. The red light emit a light intensity at 0.2 lux and if the cow perceive the red light or just a higher light intensity than 0 lux can not be determined and has earlier been questioned in several studies (Dabrowska et al., 1981; Riol et al., 1989; Phillips and Lomas, 2001).

In the red light, the cows interacted fewer times with object b (the orange cone) compared to the other objects, although each interaction lasted longer. This could be explained by the fact that if cows struggle to see an object they usually explore it for a longer time (Dannenmann et al., 1984). The color of an object is determined by the object's ability to absorb and reflect light and the color of the red light that emits short wavelength (Riol et al., 1989) maybe had an impact of the appearance of the orange cone. According to Phillips and Lomas (2001), cows react differently on different colors, which might indicate that they perceive them differently. If red light are going to be used as night light in the future it would be important to consider the colors of the equipment's in the barn such as water cups and feed stations. This must however be examined more in future studies.

5.3 Activity meter

There was no significant difference between time the cows spend lying and standing in the study, which contradicts Phillips and Arab (1998) results which suggested that cows preferred to stand up in high light intensities and lay down in low light intensities. It was a tendency for the cows to lay down for a longer time in the high light intensity, which contradicts our hypothesis, that the cows would spend longer time laying down in the low light intensities compared to the high ones.

5.4 External impacts

Some external factors that might have impacted the result of the study were human factors, as late reaction rate or poor observation ability. Another external factor that most probably had an impact was how much the cows were herded and enticed through the obstacle course. The herding and enticing was reduced with the time. External disturbance may also have impacted the results, a carpenter screwed in the wall, people looked in and let in some light, other animals where herded outside the doors and problems with the ventilation which led to slippery floor. The study was a small pilot study, including only twelve cows but as the study had a change-over design each cow was exposed for each treatment in a randomize order, which reduce the influence of external factors, as well as the order of treatment.

5.5 Recommendations

Based on the results presented in this study, cows not change their behavior until a light intensity of 0 lux, which implies that they can perceive their environment in 5 lux and probably also in 0.2 lux. This means that countries with higher night light intensity recommendations, for example Germany, France, UK and Switzerland (Dannenmann et al., 1984; Phillips et al., 2000) can lower their limits, and the producers can save energy and hence money. With this information a recommendation can also be suggested to the Swedish animal welfare act, so they can establish a requirement of a specific light intensity level as night light to dairy cows. It is however still unknown if cows can perceive red light or not, but what is suggested by Dahl (2005), that farmers using red lights are able to observe the animals without disturbing them, might be wrong. The cows can perceive their environment in red light, but if the cows perceive the red light or simply the light intensity that the red light resources emitted is still unclear.

6 Conclusion

This study shows that cows can perceive both a familiar and an unfamiliar environment in at least 5 lux and probably in darker light intensities as well (0.2 lux). Whether the cows perceived the color red or not could not be determined.

7 Acknowledgment

I would like to thank my supervisor Emma Ternman for the help and the support in my research and my assistant supervisor Sabine Ferneborg who also helped me a lot in the research, and especially the work in SAS. Furthermore I would like to thank my colleague Pernilla Olsson, who at the same time performed a physiological study on the cows, for helping me plan and perform the experiment. Finally I would like to thank the staff on Swedish Livestock Research Centre, Lövsta for all the help with the management of the cows and solving various technical problems.

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Appendix

Appendix 1. The designs of the unfamiliar obstacle courses (b-i)

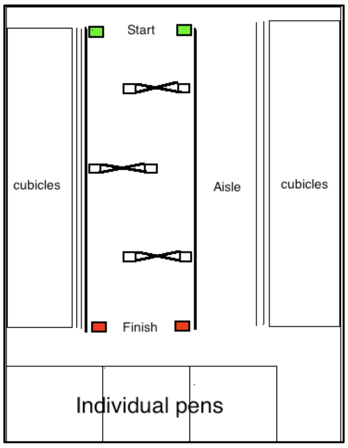

Fig. 1. The unfamiliar obstacle course design b. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

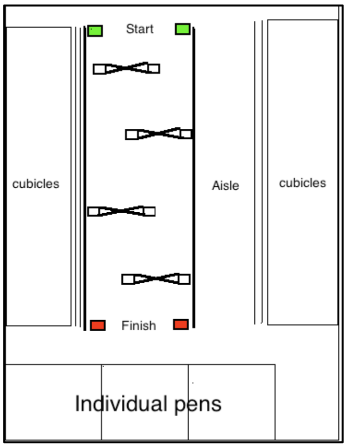

Fig. 2. The unfamiliar obstacle course design c. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

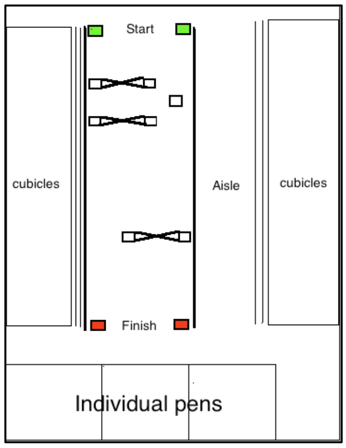

Fig. 3. The unfamiliar course design d. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted

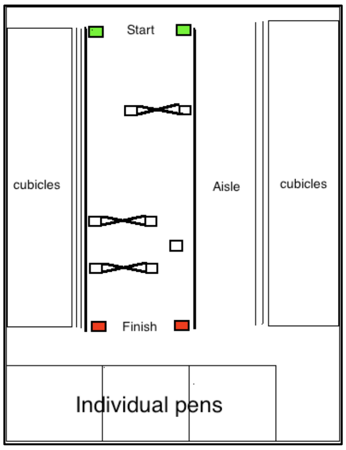

Fig. 4. The unfamiliar obstacle course design e. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

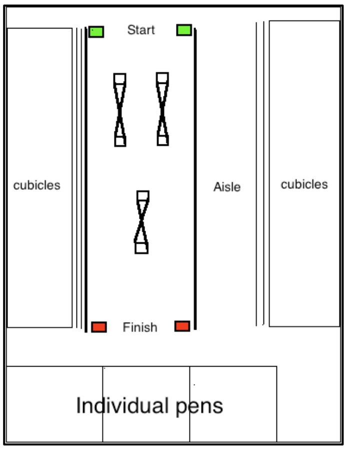

Fig. 5. The unfamiliar obstacle course design f. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

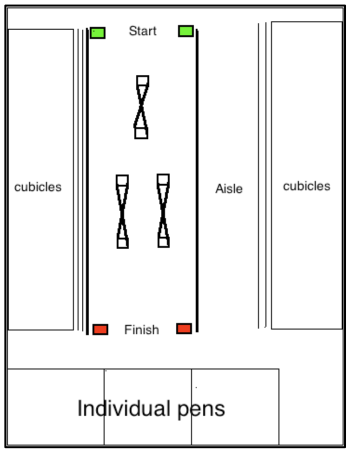

Fig. 6. The unfamiliar obstacle course design g. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

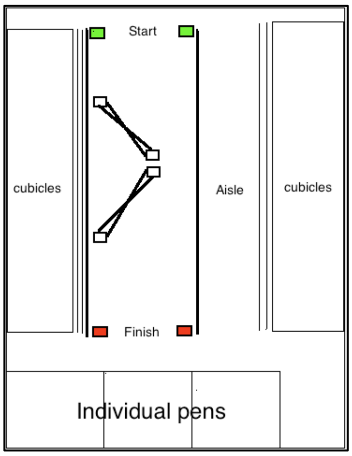

Fig. 7. The unfamiliar obstacle course design h. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

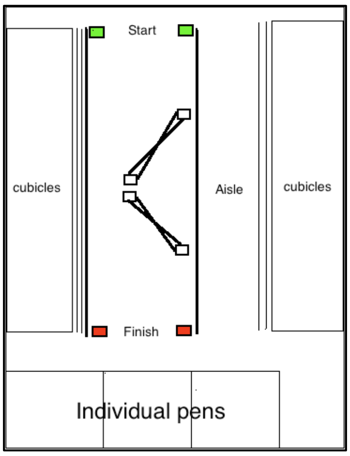

Fig. 8. The unfamiliar obstacle course design i. The crosses symbolize the obstacles of white-colored bars on plastic blocks. The red and green squares symbolize plastic blocks that were the start- and finish line. The several stripes besides the obstacle course illustrates the two slatted aisles.

Appendix 2. Picture and measurements of the objects in the Novel object test

Fig. 1. Picture of the objects (a-h) used in the Novel object test numbered a-h. a, a red plastic bag; b, a orange cone; c, a pink box turned upside down; d, a beach ball; e, a blue plastic bag; f, a umbrella; g, a swim ring; h, a pinwheel.

Tabel 1. Measurements of the objects used in the Novel object test

Number of object	**Object**	**Measurement (cm)**
a	Red plastic bag	50 x 60 x 23
b	Orange cone	28 x 28 x 51
c	Pink box turned upside down	35 x 30 x 20
d	Beach ball	Diameter 35
e	Blue plastic bag	50 x 35 x 35
f	Umbrella	Diameter 50 x height 35
g	Swim ring	Diameter 40 x height 30
h	Pinwheel	25 x 70

Appendix 3. Average values of observations in the four different light intensities in the obstacle courses

Table 1. The average values ±SE of parameters observed at the four different light intensities in the obstacle course

	0	5	20	50
Interactions with obstacles (Nº)	1.96±1.41*	1.46±1.41	1.25±1.30	1.15±1.03
Stand still (Nº)	1.10±1.41	1.21±4.24*	0.71±1.11	0.85±1.05
Interactions surrounding (Nº)	0.67±0.71	1.04±0.71	0.71±1.17	0.98±0.91
Defecation (Nº)	0.17±0.00*	0.19±0.00	0.22±0.38	0.33±0.44
Jump over obstacles (Nº)	0.17±0.00	0.23±0.00	0.21±0.41	0.10±0.42
Knock down (Nº)	0.33±0.00	0.35±0.00	0.25±0.48	0.40±0.79
Steps through obstacle course (Nº)	15.81±7.07****	14.00±1.41	13.92±2.56	13.98±2.29
Steps through the aisle (Nº)	13.21±2.32*	12.73±2.02	12.35±1.52	12.77±2.46
Time through the obstacle course (sec)	87.37±11.96**	68.39±50.67	51.55±33.60	57.61±33.23
Step rate (number of steps /time (sec)	0.30±0.11	0.27±0.16	0.37±0.20*	0.30±0.13
Step length through aisle (m)	1.16±0.07*	1.20±0.30	1.23±0.14	1.21±0.20

****p<0.001
***p<0.005
**p<0.01
*p<0.05

Table 2. . The average values ±SE of parameters observed at the presence of red light or not in the obstacle courses

	Presence of red light	No presence of red light
Interactions with obstacles (Nº)	1.50±1.18	1.42±1.33
Stand still (Nº)	0.85±1.14	1.08±1.37
Interactions surrounding (Nº)	0.99±0.97	0.71±0.78*
Defecation (Nº)	0.24±0.45	0.22±0.49
Jump over obstacles (Nº)	0.15±0.23	0.21±0.27
Knock down (Nº)	0.43±0.73*	0.24±0.57
Steps through obstacle course (Nº)	13.93±2.78	14.93±2.93
Steps through the aisle (Nº)	12.81 ±1.65	12.72±1.27
Time through the obstacle course (sec)	63.99±42.43	68.47±75.78
Step rate (number of steps /time (sec)	0.31±0.17	0.31±0.18
Step length through aisle (m)	1.19±0.16	1.20±0.13

*p<0.05

Appendix 4. Average values of observations with or without presence of red light in the Novel object test

Table 1. The average values ±SE of parameters observed at the four different light intensities in the novel object test

	0 Lux	**5 Lux**	**20 Lux**	**50 Lux**
Time to first interaction (sec)	512.40±291.62*	284.57±216.45	336.10±237.10	290.71±211.54
Interactions with objects (Nº)	2.04±2.37*	2.46±2.19	1.67±1.43	3.00±2.81
Length of interactions (sec)	4.53±15.26	5.46±23.47	3.85±14.41	4.90±21.58

*p<0.05

Table 2. . The average values ±SE of parameters observed at the presence of red light or not in the novel object test

	Presence of red light	**No presence of red light**
Time to first interaction (sec)	324.05±372.92	387.84±405.27
Interactions with objects (Nº)	2.17±1.94	2.42±2.58
Length of interactions (sec)	52.675±43.45	44.33±49.42

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