AARHUS UNIVERSITY
DEPARTMENT OF ANIMAL SCIENCE

# Behaviour of lame dairy cows in hospital pens - effect of lying surface


Anne Sandgrav Bak
Study number: 20094451

Supervisors:
Margit B. Jensen and Mette S. Herskin

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# Preface

This master thesis, concerning the behaviour of lame cows, consists of a background, a manuscript for an article and an expanded discussion. The background is a literature review describing relevant knowledge and studies regarding lame cows. The manuscript consists of an introduction, material and methods, results and discussion and will hopefully later be published. In the expanded discussion are the design, data analyses, perspective as well as my learning outcomes discussed.

Through the process of work with this master thesis, a lot of people have helped me in different ways. First and foremost, I want to thank my main supervisor, Margit B. Jensen, and my co-supervisor, Mette S. Herskin. During a busy workday, you have always been ready to answer my questions, which there have been many of, and I have really enjoyed our meetings where we have discussed and evaluated the project. I would also like to thank Lars Bilde Gildbjerg who has been an invaluable help through my work with preparing data for statistical analysis.

A big thanks to my colleges at the department of Animal Behaviour and Stress Biology. I have enjoyed having some nice and friendly colleges that made me feel like a part of the group. Especially, I would like to thank Mona Lilian Vestbjerg Larsen, Sarah-Lina Aagaard Shild and Maria Valain Rørvang for help and support through the whole process.

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# Summery

Lameness in dairy cows is a serious problem affecting welfare and productivity, with no improvement in the prevalence of lame cows over the past two decades. Hence, knowledge about management of lame cows is needed in order to mitigate the negative effects on productivity and welfare. Moving lame cows to a hospital pen may relieve the cow. However, no studies have investigated the effect of lying surface in hospital pens on the behaviour of lame cows.

One important aspect of dairy cow welfare is lying behaviour, and a significant reduction in this behaviour leads to physiological and behavioural stress reactions. The lying surfaces in free stalls affect the lying behaviour in cows, and it has been suggested that lame cows are more sensitive to the lying surface compared to non-lame cows. Thus, the aim of this study was to investigate the effect of two different lying surfaces, 30 cm deep bedded sand and 24 mm rubber mats, on the lying behaviour of lame cows housed in individual hospital pens. Thirty-two lame dairy cows with a gait score 4 on a 5 point scale (1 is normal gait, 5 is severely lame) were kept in individual hospital pens, where they were restricted to deep-bedded sand for 24 h and rubber mats for 24 h in a cross-over design. The lying behaviour was recorded for 18 h for each cow on each type of lying surface.

Lame cows showed a higher total lying time, higher frequency of lying bouts and shorter duration of lying down and getting up movements when kept on deep-bedded sand compared to rubber mats. A higher number of lame cows were lying laterally on deep-bedded sand compared to rubber mats. The duration of lying intention movements, both regarding latency time from the first intention movement until lying down and total duration of intention movements per lying bout, was shorter on deep-bedded sand compared to rubber mats.

These results suggest that lame dairy cows are more reluctant to lie down and get up on rubber mats compared to deep-bedded sand, and that rubber mats are less comfortable to lie on compared to sand, which is reflected in the lower lying time. I suggest that deep bedding of for instance sand may allow lame dairy cows rest according to their need to a higher degree than harder surfaces such as rubber.

# Sammendrag

Halthed hos malkekøer er et væsentlig velfærds- og produktionsmæssigt problem, og ingen forbedring i hyppigheden af halte malkekøer er set over de sidste 20 år. Derfor er viden om, hvordan disse dyr skal håndteres, vigtig i forhold til at kunne mindske de negative påvirkninger på velfærden og produktiviteten. Opstaldning i sygeboks kan være en måde at aflaste de halte køer. Dog har ingen studier undersøgt, hvordan underlagets beskaffenhed påvirker adfærden hos halte køer opstaldet i sygebokse.

Liggeadfærden er vigtig for velfærden hos køer, og en betydelig reduktion i liggetid kan føre til fysiologiske og adfærdsmæssige stressreaktioner. Liggeunderlaget påvirker liggeadfærden hos køer, og det er forslået, at halte køer er mere sensitive i forhold til typen af underlag end ikke-halte køer. Derfor er formålet med dette speciale, at undersøge hvordan to forskellige liggeunderlag (30 cm sand og 24 mm gummimåtter) påvirker liggeadfærden hos halte køer opstaldet i individuelle sygebokse. Toogtredive halte køer med en halthedscore 4 på en 5 point skala (1 er normal gang, 5 er kraftig halt) var opstaldet i sygebokse, hvor de stod på sand i 24 timer og gummimåtter i 24 timer i et cross-over design. Liggeadfærden blev registreret i 18 timer for hver ko på hvert underlag.

Halte køer i forsøget lå mere ned, havde et højere antal liggeperioder og brugte mindre tid på at lægge sig ned og rejse sig, når de var opstaldet på sand sammenlignet med gummimåtter. Et højere antal køer lå ned fladt udstrakt på siden på sand sammenlignet med gummimåtter. Varigheden af ligge-intentioner var lavere både med hensyn til latenstiden, fra den første intention indtil koen lå ned, og den totale varighed, når de var opstaldet på sand sammenlignet med gummimåtter.

Disse resultater tyder på, at halte malkekøer er mere tilbageholdene, når de skal lægge sig og rejse sig på gummimåtter sammenlignet med sand, og at gummimåtter er mindre behageligt for halte køer at ligge på sammenlignet med sand. Baseret på dette forslår jeg, at dybstrøelse, med for eksempel sand, til en højere grad tillader halte malkekøer at få opfyldt deres behov for hvile sammenlignet hårdere underlag som for eksempel gummimåtter.

# Background

In modern dairy production, lameness is one of the largest challenges for animal welfare, productivity and economy (Warnick et al., 2001; Galindo and Broom, 2002; Green et al., 2002; Ettema and Østergaard, 2006). For sick or injured dairy cows kept in loose housing, the opportunity to be moved to an individual hospital pen implies lower competition and the possibility to perform behaviours motivated by sickness or pain such as increased lying and isolation, which may relieve lame cows and contribute to a quicker recovery. In Denmark, recent legislation prescribes that dairy farmers must be able to house sick or injured animals in hospital pens or cubicles with dry and soft bedding (Anonymous, 2014). However, at present no studies have examined effects of the design of hospital pens on the welfare or recovery of lame cows. One important aspect of dairy cow welfare is lying behaviour (Jensen et al., 2005; Munksgaard and Simonsen, 1996). Hence, the present study compares the effect of two different lying surfaces, deep-bedded sand and rubber mats, on the lying behaviour of lame cows housed in individual hospital pens.

**Bovine lameness – definition and prevalence**

At present, several different definitions of lameness exist. In the Welfare Quality protocol, bovine lameness is defined as an abnormality of movement caused by a reduced ability to use one or more limbs in a normal manner (Welfare Quality® Consortium, 2009). Olechnowicz and Jaskowski (2011) include pain and discomfort from hoof lesions or leg injuries in their definition of lameness, described as a deviation in gait resulting from pain or discomfort from hoof or leg injuries. Based on the finding that 92 % of all cases of lameness in dairy cows is caused by lesions in the foot (Murray et al., 1996) and that lameness can be associated with pain (O'Callaghan, 2002; Rushen et al., 2007), I have chosen to use the definition by Olechnowicz and Jaskowski (2011) in this thesis.

Across countries, lameness is among the most important health problems in dairy herds (Bell et al., 2006; Capion et al., 2008; EFSA, 2009). In herds with loose housing, prevalences of dairy cows with a lameness score no less than three on a five point scale, have been reported to be between 25 and 30 % (Espejo et al., 2006; Ito et al., 2010; Thomsen et al., 2012), with the highest rates observed in herds housed in free stalls (Cook, 2003). This master thesis focus on these lame cows and how to house them regarding lying surface.

**Effects of lameness on the herd economy, productivity and cow welfare**

Bovine lameness has a well documented impact on productivity, economy and welfare (Warnick et al., 2001; Galindo and Broom, 2002; Green et al., 2002; Ettema and Østergaard, 2006). Lameness has been shown to reduce milk production for months before the cow is diagnosed and treated for lameness and for months after treatment (Green et al., 2002). Fertility is also affected by lameness. For example, Kilic et al. (2007) reported increased interval from calving to first service of 82 to 92 days and a decreased conception rate of 55 to 41 % for lame cows compared to non-lame animals. In addition, lameness may be the reason for premature culling, and lame cows have been shown to be twice as likely to be culled compared to non-lame cows (Booth et al., 2004). Based on findings as these, lameness can challenge the production economy, and the loss per first case of lameness has been estimated to be € 192 in a modern, average danish dairy herd (Ettema and Østergaard, 2006).

Several definitions of animal welfare have been proposed. One of the most well known is that of Broom. Broom (1988) defined animal welfare as the state of an individual as regards its attempts to cope with its environment. In this context, coping implies the degree of control with the mental and physical state of an individual (Broom, 1991). Duncan and Petherick, (1991) define animal welfare as the absence of strong negative feelings and the presence of positive feelings, thereby focusing on the mental state of the animal. Fraser defines animal welfare from three conceptions, the functioning of the animal, the affective state of the animal and natural living, thereby combining several welfare definitions (Fraser et al., 1997). The functioning describes the health and normal functioning of the animal, with physically healthy animals having a high welfare. The affective state of the animal concerns the feeling and emotions of the animal, were a high welfare depends on the freedom from pain, fear, hunger and other negative states. The last one is natural living, concerning the naturalness of the environment the animals are kept in and the ability of an animal to perform natural behaviour. Using this definition lameness is a welfare problem because lame cows are in pain (affective state), they have a lower milk production and reproduction (the functioning of the animal) and they have a reduced mobility and reduced oestrous behaviour (natural behaviour) (von Keyserlingk et al., 2009). Irrespective of the welfare definition used, bovine lameness is a welfare problem, the magnitude of which depends on e.g. the discomfort and pain associated with the conditions as well as the impact on the behaviour of the lame cows. Minimizing pain is therefore a key concept in improving the welfare of lame cows, hence knowledge about how to relieve and handle lame cows is needed.

**Causes for lameness**

*Common diagnoses causing lameness*

As mentioned, bovine lameness is deviations in gait resulting from pain or discomfort due to hoof or leg injuries (Olechnowicz and Jaskowski, 2011). Hence, the term bovine lameness covers several diagnoses located in the limbs of cattle, e.g. in horn or skin of the hoof (Olechnowicz and Jaskowski, 2011; Thomsen et al., 2012). Digital dermatitis is one of the most common infectious skin lesions in dairy cows and white line disease and in particular sole ulcers are the most common non-infectious horn lesions (Murray et al., 1996; Hedges et al., 2001; Thomsen et al., 2012).

The term sole ulcer defines lesions located in the region of the sole/bulb junction. Sole ulcers most often affect one or both lateral hind claws in high-yielding dairy cows. The lesions cause damage to the dermis resulting in haemorrhage and necrosis (Amstel and Shearer, 2006). As do sole ulcers, white line disease normally affects one or both lateral hind claws in high-yielding dairy cows. This condition is characterized by haemorrhage in the white line or a separation of the sole from the abaxial sidewall of the hoof. This type of lesion is most often found in the apical portion of the sole on the abaxial border (Booth et al., 2004; Sanders et al., 2009) (Figure 1). Thomsen et al. (2012) found that first parity cows are at higher risk of suffering from skin lesions, whereas older cows are at higher risk of suffering from horn lesions. In the experimental part of this thesis only cows diagnosed with sole ulcers or white line disease were included. One fourth of the cows were in their first lactation, 50 % in their second and 25 % in their third or later lactations.

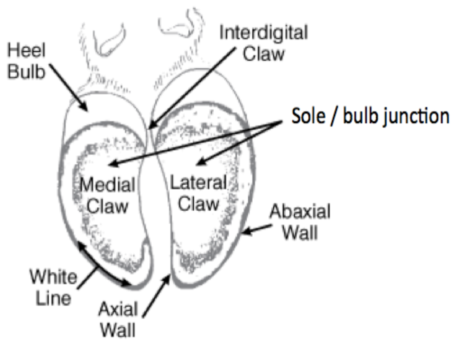


Figure 1: Ventral view of a bovine hoof showing selected anatomical locations. (The Alberta Dairy Hoof Health Project)

**Risk factors for the development of bovine lameness**

Even within single diagnoses, bovine lameness is multifactorial and has several underlying risk factors. For horn lesions important risk factors are nutrition, calving, external trauma and infectious agents (Cook and Nordlund, 2009). Nutritional factors affect the development of horn lesions through the maintenance of horn quality and via the link to ruminal acidosis, which may increase the risk of laminitis (Cook et al., 2004b). Laminitis is a disturbance of the microstructure of the corium, which is the horn producing tissue of the claw (Nocek, 1997). The risk of horn lesions is increased during the period around calving, due to the changes in feed, housing, hormones and behaviour typically occurring in relation to calving and these changes may be associated with weakness of the connective tissue, making the hoof more vulnerable to external stresses such as concrete flooring or a hard lying surface (Webster, 2001; Tarlton et al., 2002; Cook and Nordlund, 2009). Factors that can lead to external trauma, for example sharp edges, as well as infectious agents, increasing the risk of infectious conditions such as digital dermatitis, are other risk factors for injuries to the bovine hoof (Capion et al., 2008; Cook and Nordlund, 2009). Another factor influencing the risk of exacerbating damages to the claw is cow comfort (Cook and Nordlund, 2009). Here, walking surface is an important contributor. Concrete flooring exacerbate the development of horn lesions and several studies have reported a higher prevalence of lameness in free stall herds compared to tie stall herds, a finding which have been suggested to be caused by increased exposure to concrete walking surfaces in free stalls (Bergsten and Herlin, 1996; Cook, 2003; Cook and Nordlund, 2009). Surface hygiene and dryness also play a role in relation to hoof weakness and infectious diseases which may cause lameness (Borderas et al., 2004; Cook and Nordlund, 2009). Hence, keeping alleyways as clean and dry as possible can reduce the incidence of lameness (Blowey, 2005). The behaviour of cows is another important factor, which plays a role in the development of hoof lesions and lameness. In a study with 35 first parity cows, a reduced lying time exacerbated the development of horn lesions. The cows were housed in an overstocked cubicle building just after calving, and due to the overstocking, lying time was reduced to an average of 10 h per day, but some animals were lying as little as 5 h. The decreased lying time was positively related to the incidence of severe hoof lesions and lameness for up to four months after calving (Leonard et al., 1996). A consequence of the decreased lying time is the increased time spent standing, which is also positively correlated with the incidence of lameness (Galindo and Broom, 2000). This is especially affecting low-ranking cows, since they spend less time lying and more time standing compared to older higher-ranking animals due to competition for resources (Galindo and Broom, 2000). An increased daily standing time, for example during milking or stall use, can therefore also influence the incidence of lameness (Cook and Nordlund, 2009). Standing with only the front hooves in the free stall, also termed perching, is another risk behaviour which is positively correlated to lameness (Galindo and Broom, 2000). Thus, lameness can be triggered when the cow is exposed to one or more lesion triggering factors and aspects of cow behaviour may exacerbate the severity of these lesions. Due to lameness being a multifactorial condition with a wide range of risk factors, eliminating lameness will be very difficult. This is also reflected in the high prevalence, which has not declined significantly within the last 20 years (Clarkson et al., 1996; Barker et al., 2010; Thomsen et al., 2012). Hence, knowledge about management of lame cows is needed in order to mitigate the negative effects of the condition and improve the welfare.

**Lameness affects the behaviour of dairy cows**

The risk of lameness can be affected by behaviour, but lameness can also affect the behaviour of cows (Cook and Nordlund, 2009). Lame cows may experience pain (O'Callaghan, 2002; Rushen et al., 2007). However, in cattle, behavioural signs of pain may be subtle, why it can be hard to identify a cow in pain, until the condition is at an advanced stage (Weary et al., 2006). Still, detailed behavioural analyses have shown that some changes can be detected even in the mild stages of lameness. Cows will alleviate the discomfort and pain associated with lameness through changes in posture and gait, thereby minimizing the weight bearing on the affected limb e.g. by arching of the spine, hanging and nodding of the head as the animal walks, shortening or lengthening of the stride on the affected limb and repeatedly lifting the affected limb from the ground when the cow is not moving (Whay, 2002). These changes are often included in locomotion scoring systems used to detect and score the level of lameness in cows (Flower and Weary, 2006; Thomsen et al., 2008).

Furthermore, there are also some general changes in the behaviour of lame cows. Lame cows spend more time lying (Chapinal et al., 2009; Ito et al., 2010; Thomsen et al., 2012), less time feeding (González et al., 2008; Gomez and Cook, 2010), are less active than non-lame cows (O'Callaghan et al., 2003; Walker et al., 2008), and  are less aggressive as well as defeated in aggressive interactions to a larger extend than healthy animals (Galindo and Broom, 2002). In addition, lame cows change position from standing to lying less frequently (resulting in a lower number of daily lying bouts) and have longer and more variable lying bouts compared to non-lame cows (Chapinal et al., 2009; Gomez and Cook, 2010; Ito et al., 2010; Thomsen et al., 2012). Furthermore, lame cows spend more time standing still in the free stalls (Cook et al., 2004a), less time walking and show less oestrous behaviour (mounting, chin resting, sniffing and flehmen) (Walker et al., 2008). However, findings regarding behavioural effects of lameness are not always consistent, since Yunta et al. (2012) did not find a difference in total lying time or number of lying bouts, when comparing lame versus non-lame cows and some studies have even reported a lower lying time for lame cows compared to non-lame cows on some lying surfaces (Cook et al., 2004a; Gomez and Cook, 2010).

Most studies of behavioural consequences of lameness have not taken the underlying pathological condition into account. Thomsen et al. (2012) found that lame cows diagnosed with a skin lesion showed the typically described changes in lying behaviour (increased lying time and increased duration of lying bouts), whereas lame cows diagnosed with horn lesions did not. Contrarily, Chapinal et al. (2009) found that cows with sole ulcers had a longer daily lying time due to longer lying bouts, and that cows diagnosed with digital dermatitis and haemorrhages did not differ from non-lame cows regarding their lying behaviour. Thus, even though results are not unambiguous, the available knowledge suggests that the underlying pathology should be taken into account when behavioural changes induced by lameness are examined. As mentioned, only cows diagnosed with sole ulcers or white line disease were included in the experimental part.

In addition to the underlying pathology, factors such as resting environment (including lying surface) affect the degree to which the behaviour is modified by lameness (Ito et al., 2010). These authors showed that severely lame cows increased the daily lying time compared to healthy cows when housed in free stalls with sand or sawdust bedding, but no differences were found between lame and healthy cows when housed in free stalls with geotextile mattresses. Thus, the lying surface has significant impact on the lying behaviour of lame cows.

Based on the above mentioned findings regarding lameness, pain and behaviour, it has been suggested that lame cows are less able to cope with their environment (Galindo and Broom, 2002). In order to safeguard the welfare of lame cows, we need to be aware of these changes and adjust the environment accordingly. Moving a lame cow to a hospital pen with less competition for food or a lying place and provided with soft bedding in the lying area, may be one solution to this. In Denmark, dairy farmers must be able to house sick and injured animals in hospital pens with dry and soft bedding (Anonymous, 2014). However, at present no further specifications regarding dryness and softness have been formulated. Several studies have investigated the effect of lying surface on the behaviour of lame and non-lame cows in tie stalls and free stalls, but until now there is a lack of studies examining the effect of lying surface on the behaviour of lame cows kept in hospital pens. Below, the present knowledge about relations between lying behaviour and animal welfare as well as lying behaviour and lying surface in the home environment of dairy cows will be reviewed.

**Lying behaviour as an indicator of welfare**

In the last decades studies have shown the importance of lying behaviour for cattle and today lying is considered a behavioural need (Munksgaard and Simonsen, 1996; Jensen et al., 2005). Cattle will increase their work to maintain access to a lying place and operant conditioning studies have shown that they will work increasingly hard to defend access to lie down for approximately 12 h/d (Jensen et al., 2005). Furthermore, lying may dominate other basic motivations such as eating after only a few hours of forced standing (Metz, 1985). However, there is a wide range in lying time within otherwise healthy dairy cows. Non-lame cows lie down for between 8 and 16 hours per day distributed between 15 to 25 lying bouts. The duration of these bouts may vary from a few minutes to more than 3 h (Krohn and Munksgaard, 1993). The large variation in lying behaviour can, as mentioned previously, be a result of factors such as housing system, amount and type of bedding, flooring and stocking density. Thus, lying behaviour reflects the environment of the cows. In general, it has been suggested that dairy cows have a demand to lie down for at least 12 hours per day, and a total lying time below 10 hours has been shown to lead to physiological and behavioural stress responses (Munksgaard and Simonsen, 1996). Different measures of lying behaviour have been suggested to be indicators of sufficient lying or the ease of which the cows can perform lying behaviour and as such, welfare (Plesch et al., 2010). Examples of measures related to animal welfare are total lying time (Haley et al., 2000; Fregonesi and Leaver, 2001), number and duration of lying bouts (Haley et al., 2000), duration of the lying-down and getting-up sequences (Whay et al., 2003), as well as interruptions in these and deviations from the normal lying-down or getting-up sequence (Krohn and Munksgaard, 1993; Wechsler et al., 2000; Welfare Quality® Consortium, 2009). Accordingly, lying behaviour is part of the Welfare Quality assessment protocol for dairy cattle used to evaluate animal welfare on farms (Welfare Quality® Consortium, 2009).

**Relation between lying behaviour and lying surface in the home environment**

*Non-lame cows*

Effects of lying surface on the behaviour of cows are well described. Tucker and Weary (2004) showed that cows increased the time spent lying, increased the number of lying bouts and decreased time spent perching, when kept in free stall housing with well bedded mattresses (7.5 kg sawdust) compared with less bedded mattresses (0 or 1 kg sawdust). In addition, the cows preferred the well bedded mattresses when given the choice (Tucker and Weary, 2004). In a study by Haley et al. (2001), cows housed in tie stalls on mattresses increased their total lying time by 1.8 h per day, had a higher number of lying bouts and shorter duration of individual lying bouts, compared to cows housed in tie stalls with a concrete floor. Similarly, non-lame cows were shown to spend more time lying on comfort rubber mats (thickness 21 mm), compared to standard rubber mats (thickness 15 mm), to increase lying on rubber mats compared to concrete flooring, and when given the choice, they preferred comfort mats (Herlin, 1997). Thus, cows prefer to, and spend more time lying on a soft surface compared to a harder surface.

However, the lying surface is not only affecting the lying time and lying-dynamics but also the duration of intention movements (Lidfors, 1989). One such intention movement is examining the surface by placing the head close to the surface and sniffing it. This may be accompanied by moving the head over the surface from side to side in an oscillating manner, the degree of which depends on the lying surface. If a cow is housed on a harder surface, she may spend more time engaged in oscillating behaviour before lying down in comparison when housed on a softer, well bedded surfaces, as reported by Müller et al. (1989). However, these results were confounded by tethering, limiting the ability to conclude whether the lying surface or the tethering as such influenced the behaviour. Tucker and Weary (2004) observed a significant drop in the occurrence of the oscillating behaviour per lying bout when cows were housed on a well-bedded mattress compared to a less bedded mattress. Thus, even though not all studies have been able to find effects of floor type on the occurrence of intention movements, (e.g., Krohn and Munksgaard (1993) did not find a difference in time spent performing oscillating movements when comparing tethered cows on concrete floor covered with a small amount of straw versus tethered cows on rubber mats with straw), the degree of oscillating and sniffing movements is reflecting the cows' perception of the lying surface. The quality of the lying surface is not the only factor affecting the lying behaviour. Familiarity with the surface material has also been shown to have an effect, since Tucker et al. (2003) found that lame cows increased their lying time on sand after a period of familiarization.

Overall, non-lame cows prefer a soft lying surface, lie down for longer and have fewer interruptions of the lying-down sequence when housed on a soft lying surface. In addition, non-lame cows use less time on pre-lying intention movements when housed on a soft surface. Factors such as familiarity with the lying surface affect the cow's preferences and behaviours as well and should be taken into account in examinations of effects of lying surface on lying behaviour of cattle.

*Lame cows*

Until now, only a few studies have examined effects of the lying surface on the behaviour of lame cows, and to the best of my knowledge, all reports have been carried out using group-housed animals kept in free stalls. Cook et al. (2004a) compared time budgets of lame vs. non-lame cows kept on sand vs. rubber crumb-filled mattress surfaces. When the cows were kept in sand filled stalls, lame and non-lame cows were shown to lie down for a comparable duration of time (12 h / day). However, lame cows housed on rubber crumb-filled mattresses spent more time standing and showed an overall reduction in daily lying time (10 h / day) compared to non-lame cows (12 h / day). In agreement, Gomez and Cook (2010) found no difference in lying time between lame vs. non-lame cows housed in stalls filled with sand, but a lower lying time and increased time spent standing for lame cows housed in stalls with rubber crumb-filled mattresses compared to non-lame cows kept in stalls with rubber crumb-filled mattresses. Contrary to this, Ito et al. (2010) found that severely lame cows in deep bedded (sand or sawdust) free stalls had a higher daily lying time and longer lying bouts compared to non-lame cows. This difference was not seen between lame and non-lame cows kept on geotextile mattresses. When Ito et al. (2010) compared severely lame cows on deep bedding with cows kept on mattresses, only the lying time, but not the lying bout frequency or average bout duration differed. It has been suggested that the reduced lying time, as reported in some studies, for lame cows kept in stalls with mattresses, compared to sand-filled stalls, can be explained by the ease of getting up and lying down (Cook, 2009). Sand provides cushion, traction and support, which may make it easier for a lame cow to get up and lie down. In contrast, the use of a firm mattress for getting up/down may be associated with pain due to the limited contact point between the sore foot and the firm mattress (Cook, 2009). However, this hypothesis has not been tested. Hence, even though the underlying mechanism has not been clarified, the lying behaviour of lame cows housed in free stalls is affected by the lying surface, with increased lying time on softer lying surfaces.

**Aim**

The aim of this experiment was to investigate effects of sand versus rubber as a lying surface in individual hospital pens on the lying behaviour of lame dairy cows as part of the study of the behaviour and welfare of lame dairy cows. This study involved comparison of 30 cm of deep bedded sand versus 24 mm rubber mats and 32 cows, with a lameness score 4, were kept for 18 hours in purpose-made individual hospital pens. All cows experienced sand as well as rubber mats in a balanced order. I expected that the cows when kept on the deep bedded sand would show a higher lying time and a longer duration and a higher frequency of lying bouts compared to when kept on rubber mats. Furthermore, I hypothesized that cows when kept on the sand would show increased ease of lying down and getting up, indicated by a shorter duration of these behavioural sequences. In addition, I expected a lower duration of lying intention movements and fewer lying interruptions on the deep bedded sand compared to the rubber mats.

*Overview of hypotheses*

1. Lame cows kept on deep bedded sand have longer total lying time compared to when kept on rubber mats.
2. Lame cows have a longer duration and a higher frequency of lying bouts when housed on deep bedded sand compared to rubber mats.
3. When kept on deep bedded sand, lame cows lie down and get up more quickly than when kept on rubber mats.
4. The duration of lying down intention and lying down interruptions are shorter when the cows are kept on deep bedded sand compared to rubber mats.

# Manuscript

# Effect of sand and rubber surface on the behavior of lame cows in hospital pens

Anne S. Bak, Mette S. Herskin and Margit B. Jensen

*Aarhus University, Department of Animal Science, AU-Foulum, Blichers Allé 20, DK-8830 Tjele, Denmark*

**Abstract** *To be submitted to Journal of Dairy Science*

In dairy cows, lameness is a serious problem affecting the welfare and productivity. Hence, knowledge about management of lame cows is needed in order to mitigate these negative effects. Moving lame cows to a hospital pen may relieve the cow. However, no studies have investigated the effect of lying surface of hospital pens on the behavior of lame cows. This study investigates the effect of a deep bed of 30 cm sand versus 24 mm rubber mats as lying surfaces in individual hospital pens on the lying behavior of lame cows. Thirty-two lame dairy cows were kept in individual hospital pens, with either deep-bedded sand or rubber for 24 h in a cross-over design. On each type of lying surface, the lying behavior was recorded for 18 h for each cow. Keeping the lame cows on a deep-bed of sand compared to rubber mats led to a higher total lying time, higher number of cows lying laterally, higher frequency of lying bouts, shorter duration of lying down and getting up movements and a shorter duration of lying intention movements both regarding latency from the initial intention movement until lying down and the total duration of intention movements per lying bout. These results suggest that lame dairy cows are more reluctant to lie down and get up on rubber compared to sand, which is also reflected in the lower lying time. We suggest that deep bedding of for instance sand may allow lame dairy cows to rest according to their need to a higher degree a harder surface such as rubber.

# Introduction

In modern dairy production, lameness has marked negative consequences on productivity and animal welfare (Warnick et al., 2001; Green et al., 2002; Bruijnis et al., 2010). For years, lameness has been a serious problem, with no significant reduction in the reported prevalences (20-40%) during the past two decades (Clarkson et al., 1996; Espejo et al., 2006; Barker et al., 2010; Thomsen et al., 2012).

Lameness in dairy cattle is a welfare problem due to the pain associated with lameness (Rushen et al., 2007), the consequences lameness has on the functioning of the cow such as reduces milk production and reproduction (Green et al., 2002; Kiliç et al., 2007) and also due to lameness affecting their behavior such as a reduced occurrence of estrous behavior and reduced mobility (Fraser et al., 1997; Galindo and Broom, 2002; Walker et al., 2008; von Keyserlingk et al., 2009). Hence, knowledge about management of lame cows is needed in order to mitigate the negative effects on productivity and welfare. For lame dairy cows kept in loose housing being moved to a hospital pen typically implies lower competition and potentially a comfortable resting place, which may relieve the animals and contribute to a faster recovery (Weary et al., 2009). In Denmark, recent legislation prescribes that dairy farmers must be able to house sick or injured animals in hospital pens with dry and soft bedding (Anonymous, 2014). However, at present no studies have examined the effect of the lying surface in hospital pens on the behavior, welfare or recovery of lame cows.

Several studies have investigated how the lying surface influences behavior of clinically healthy cows, where a soft lying surface has been shown to increase lying time and decrease standing (Tucker and Weary, 2004; Herlin, 1997). However, only a limited number of studies have investigated effects of lying surface on the behavior of lame cows and these have, to the best of our knowledge, been carried out in loose housing with free stalls. In general, these studies show that lame cows lie down for longer and spent less time standing in the free stalls, when the stalls are deep-bedded with sand or sawdust compared to geotextile mattresses or rubber crumb-filled mattresses (Cook et al., 2004; Gomez and Cook, 2010; Ito et al., 2010). When comparing lying behavior of healthy versus lame cows kept on geotextile mattresses or rubber crumb-filled mattresses, the lame animals lie down less than the healthy controls (Cook et al., 2004; Gomez and Cook, 2010). Further, Hernandez-Mendo et al. (2007) reported a decrease gait score for lame cows moved from free stalls with sand as bedding to pasture for 4 weeks, where no decrease in gait score was found in the cows who remained in the free stalls. Hence, lame cows are sensitive towards the lying surface, which may affect their behavior as well as recovery.

One important aspect of dairy cow welfare is lying behavior (Munksgaard and Simonsen, 1996). Measures such as total lying time, duration of the lying down or getting up movement, number of lying interruptions and the frequency and duration of lying intentions are indicators which can be used to access cow comfort and welfare and these indicators have also been used to evaluated lying surfaces for dairy cows (Krohn and Munksgaard, 1993; Haley et al., 2001; Herlin, 1997; Tucker and Weary, 2004). Lying behavior is particularly useful as an indicator, because cows are highly motivated to lie down and a significant reduction in this behavior leads to physiological and behavioral stress reactions (Metz, 1985; Munksgaard and Simonsen, 1996; Jensen et al., 2005; Weary et al., 2006). Thus, the present study combined the need for knowledge about lying surfaces in hospital pens for lame dairy cows with existing knowledge regarding lying behavior of dairy cows and aimed to compare effects of two different lying surfaces on the lying behavior of lame cows when housed in individual hospital pens with a surface of either deep-bedded sand or rubber mats. The study involved thirty-two lame dairy cows with white line disease or sole ulcers at minimum one leg. We hypothesized a lying surface of deep-bedded sand in individual hospital pens would lead to a longer lying time, and longer duration as well as higher frequency of lying bouts compared to a surface of rubber mats. Furthermore, we hypothesized that cows when kept on deep-bedded sand would show increased ease of lying down and getting up, indicated by a shorter duration of these behavioral sequences. In addition, we expected a lower duration of lying intention movements and fewer lying interruptions on sand compared to the rubber mats.

## Materials and methods

**Animals and housing**

The experiment was carried out from September to December 2013 in the resident barn at AU- Foulum, Aarhus University, Denmark, housing 125 year cows.

Prior to the experiment cows were loose housed in a barn were the free stalls were fitted with mattresses (Fremtidens-staldinventar, A/S., Langå, Denmark) and the flooring in the alleys outside the free stalls was made of slatted concrete. The cows were milked twice daily in a herringbone-milking parlor and they had free access to a total mixed ration (TMR) with forage to concentrate ratio of 60:40 (% DM basis). For lactating cows the stocking density of free stalls was at least one free stall per cow and at maximum 2 cows per feeding space. Regarding dry cows the stocking density of both free stalls and feeding space were one free stall and one feeding space per cow. None of the cows had previous experience with sand as bedding.

**Inclusion and exclusion criteria**

Forty lame cows were used in the experiment. The cows were selected by weekly gait scoring of cows in the herd. The same person throughout the experiment gait scored the animals. Lactating cows were gait scored when returning from afternoon milking, while dry cows were gait scored in the dry cow pen. In order to be included as a lame cow, a gait score of no less than 4 on a 5-point scale was needed (Table 1; Thomsen et al., 2008) on the day before they were inserted in the experimental hospital pens. If there were less than 4 lame cows in one week, healthy and non-lame cows (gait score=1) were selected and included in the experiment to ensure a constant social environment in the barn. The position of lame and healthy cows in the pen was balanced. The day the lame cows were moved to the experimental hospital pen, they were first inspected in a hoof-trimming chute. When in the chute, the condition causing lameness was diagnosed and only cows diagnosed with white line disease or sole ulcers on minimum one hoof were selected. The two diseases were chosen because they are noninfectious, and not treated with analgesics. Cows diagnosed with sole hemorrhage were not used in the experiment unless they were also diagnosed with either white line disease or sole ulcers. All cows in the experiment had to be more than ±14 days from calving and to be free of any clinical signs of disease or medical treatment except the above mentioned.

Eight of the cows initially included in the study had to be excluded from the experiment because they had a diagnose different from the above described (n=2), got injured during the cause of the experiment (n=1), or fell from a gait of score 4 at the start of the experiment to a score of 1 or 2 at the end of the experiment (n=5). Cows with a score of 1 or 2 were excluded in order to avoid any uncertainty as to whether they were scored too high at experiment initiation. Thus, data from 32 lame cows were included in the present study. Of the 32 experimental cows were 8 first parity cows, 15 second parity cows and 9 third, or later, parity cows. Thirteen of the cows were in their early lactation (0-120 days in milk (DIM)), 16 in late lactation (120-414 DIM) and 3 were dry cows. The average weight of the cows when moved to the experimental hospital pen was 634 (range 505-866) kg and none of the cows had fever

(<39.5 °C) with an average rectal temperature on the first and second day in the experimental hospital pen on 38.3 (range 37.7-39) °C.

**Table 1.** Description of the 5-point ordinal gait scoring system for dairy cows used in the experiment (Thomsen et al., 2008). All cows were given a gait score 4 the day before they were moved to the test pen

| **Score/level** | **Description of level** |
|---|---|
| 1. Normal | The cow walks normally. In most cases, the back is flat, both when the cow is standing and when walking. No signs of lameness or uneven gait. No signs of uneven weight bearing between legs. No signs of head bob when the cow is walking. |
| 2. Uneven gait | The cow walks (almost) normally. In most cases, the back is flat when the cow is standing, but arched when walking. No signs of head bob when walking. The gait might be slightly uneven and the cow may walk with short strides, but there are no evident signs of lameness. |
| 3. Mild lameness | Abnormal gait with short strides on 1 or more legs. In most cases, the back is arched both when the cow is standing and walking. In most cases, there are no signs of head bob when walking. In most cases, an observer will not be able to tell which leg is affected. |
| 4. Lameness | The cow is obviously lame on 1 or more legs. An observer will in most cases be able to tell which leg is affected. In most cases, the back is arched both when the cow is standing and walking. In most cases, head bob will be evident when walking. |
| 5. Severe lameness | The cow is obviously lame on 1 or more legs. The cow is unable, unwilling, or very reluctant to bear weight on the affected leg. In most cases, the back is arched both when the cow is standing and walking. In most cases, head bob will be evident when walking. |

**Experimental design**

During the experiment, each cow was kept individually for six days in an experimental hospital pen, constructed for the purpose of the experiment (Figure 1). Four similar pens were used each holding one animal per week. The pens were 6 x 4.5 m and contained a feed trough and 4 water cups, placed centrally in the pen. Within the experimental room of the barn, the placing of each of the 4 pens allowed the experimental cows visual contact with the 3 other experimental cows of that week and physical contact with 2 companion animals. In addition, each experimental cow could get physical contact with the experimental cow kept in the neighboring pen. The cows were fed TMR (same mixture as before the experiment) for *ad libitum* intake. The cows were milked and fed twice a day, in the morning between 0600 h and 0700 h and in the afternoon between 1600 h and 1700 h.

Each experimental hospital pen had two equally sized and equipped parts, with type of surface being the only difference: one part with a deep bed of 30 cm of sand with a mean grain size 0.322 mm (‘Kosand’, Dansand, Brædstrup, Denmark) and one with rubber consisting of 19 mm mats with 5 mm studs (Kura Flex, Kraiburg, Tittmoning, Germany). The two parts were divided by the feed manger and rail on each side of this manger. Artificial light was turned on from 0600 h to 2200 h and a dim-light during the remaining of the 24 h period to enable data collection from video recordings.

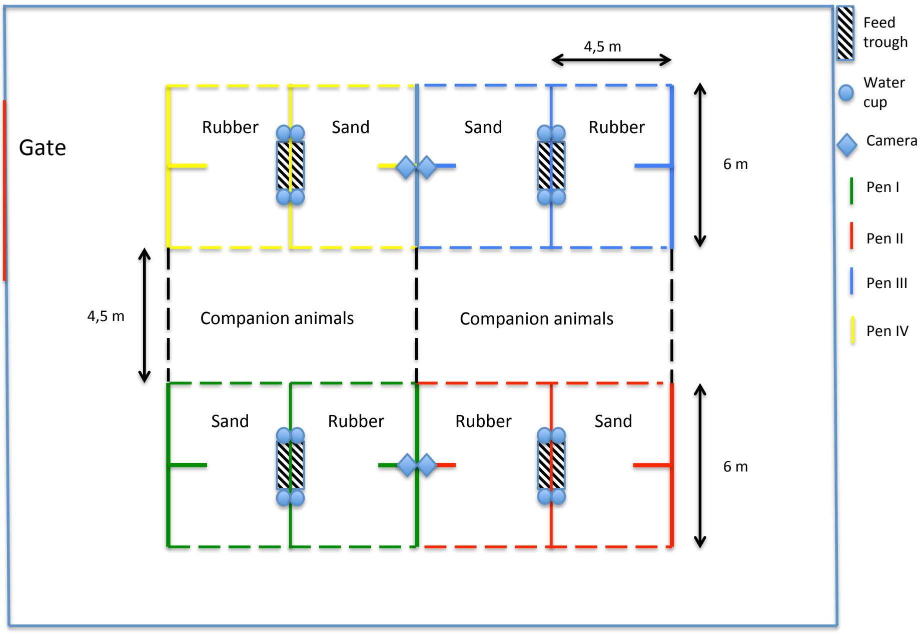


**Figure 1.** Floor plan of the experimental room of the barn with the four hospital pens used for the lame cows. Each pen was divided into two identical parts, with type of flooring as the only difference. One part was bedded with 30 cm sand and the other part had 24 mm rubber mats on the floor.

## Experimental procedures

This experiment was carried out as a cross-over design with two treatments, a deep bed of 30 cm of sand as lying surface and 24 mm rubber mats as lying surface. The experiment was running as weekly blocks. Each week (block), 4 cows were introduced to the experimental hospital pens at 1030 h. They were restricted to one half of the pen with either deep-bedded sand or rubber mats. In each block two cows were restricted to sand for the first 24 h and to rubber for the subsequent 24 h, while the other two cows were restricted first to rubber and then to sand. Due to the position of the entries to the pens, cows in pen I and II were always restricted to rubber on day 1, and cows in pen III and IV were always restricted to sand on day 1. All cows spent 24 h on each surface. Each 24-h period, the cows were milked and fed twice (0600 h and 1600 h) and the pens were cleaned and the sand leveled out. During morning milking, rectal temperature of the cows was measured and feed leftovers were weighted. On day 2 at 1030 h, the cows were moved to the other surface until the next day at

1030 h, where they were allowed access to both surfaces, as part of another experiment investigating lame cows' preferences regarding surface and social contact (Jensen et al., 2014). They were kept in the pens until Monday the week after (seven days after the first lameness scoring) where the cows were taken out of the test pen and lameness scored again. An overview of the experimental procedures during an experimental week can be seen in Figure 2.

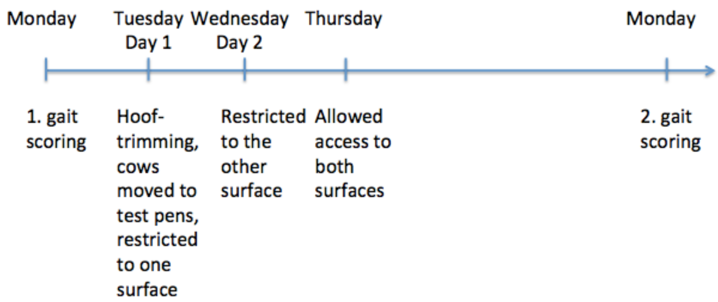


**Figure 2.** Overview, showing the experimental procedures during an experimental week (block) of the experiment. In each block, 4 cows were introduced to the experimental hospital pen after being gait scored and diagnosed. After a week the cows were gait scored a second time and moved back to the home environment

**Behavioral recordings**

Postures and behaviors of the cows were video recorded during the entire stay in the experimental hospital pens. One camera (MONACOR, TVCCD-624, Bremen, Germany) was fitted above each pen allowing a side view of the whole pen including the two parts with different lying surface. Video recordings (MSH Video, M, Shafro and Co, Riga, Latvia) of the 32 lame cows were conducted by one observer by use of focal sampling and continuous recording (Martin and Bateson, 2007). The observer had an intra-observer reliability of 99.9% agreement for total lying time, 98.8% agreement for the variable sniffing/oscillating movement and 88.2 % agreement for the getting up movement (see Table 3 for description of the variables). Agreement was calculated as correlation (Microsoft Excel, Microsoft Corp., Redmond, WA). Video recordings from 1200 h to 0600 h (18 h, initiated approximately 1.5

hours after introduction to the pens) the following morning were included for each cow and type of lying surface. An ethogram is shown in Table 2. A list of the variables calculated for each cow and type of lying surface is shown in Table 3.

**Table 2**. Ethogram of cow postures and behaviors recorded during the 2 x 18 h experimental periods in the hospital pens. Modified from [1]Plesch et al. (2010), [2]Krohn and Munksgaard (1993) and [3]Niss et al. (2009).

| Posture | Definition |
|---|---|
| Lying down, sternally | The cow is lying on sternum. Initiated when the hindquarter of the cow had fallen down and the cow had pulled the front legs from underneath the body[1]. Terminated when the cow stood up with all four legs stretched. |
| Lying down, laterally | The cow lied flat on the side, with the head resting on the lying surface[2]. Intervals of less than 5 seconds were ignored. |
| Standing | The cow was standing with body supported by four legs. |
| **Behaviors registered when the cow was standing** | |
| Sniffing | The muzzle of the cow was orientated towards the ground and closer than 10 cm from the floor. Intervals of less than 3 seconds were ignored. |
| Oscillating | The muzzle of the cow was closer than 20 cm from the ground and head was swinging at least three times away from the center of the body in a continuous and oscillating movement. |
| Kneeling | The cow fell down on one or both knees. |
| Other or no activity | None or other activities than the ones described above. |
| Not visible | The cow is standing in an angle to the camera that made it impossible to see its behavior. |
| **Behaviors and postures registered when the cow was lying sternally** | |
| Changing side | The cow changed lying side without standing up. |
| Right side | The right shoulder and right flank was closest to the ground. |
| Left side | The left shoulder and left flank was closest to the ground. |
| Breastbone raised | The cow moved the head and neck upwards and forwards and rose onto the breastbone and elbows, followed by standing[3]. |

## Data analysis

*Data editing*

Variables for each cow on each surface were calculated as described in Table 3, resulting in a data set with two observations per cow, one for each surface, for all the variables. Regarding lying intentions, two variables were calculated. One variable is the oscillating head movement and the other one is were sniffing and oscillating head movements are combined into one variable (sniffing/oscillating).

**Table 3**. A list of the variables used in this study of behavior of dairy cows when kept in hospital pens with different floor types. Each variable was calculated for each cow on each lying surface yielding 32 x 2 observations. Modified from [1]Lidfors (1989), [2]Krohn and Munksgaard (1993) and [3]Plesch et al. (2010).

| **Behavior** | **Description** |
|---|---|
| Lying time and frequency | |
| Total lying time | Initiates when the cow is lying and terminates when the cows is standing. |
| Duration (h/18 h) | Summarized duration of the time lying down. |
| Lying laterally | Initiates when lying down laterally and terminates when lying down sternally. |
| Number of cows | Has the cow been lying laterally; yes or no. |
| Lying bout | Initiates when the cow is lying down and terminates when the cow stands. |
| Duration (min/bout) | Mean duration of the lying bouts. |
| Frequency (n/18 h) | Number of lying bouts. |
| Lying down movements | |
| Lying down interruptions | The cow kneels but the lying down sequence is interrupted[2]. |
| Number of cows | Has the cow been making any interruptions; yes or no. |
| Lying down movement | From the initiation of kneeling until the cow is lying[2]. |
| Duration(s) | The median of the duration of the lying down movements. |
| Getting up movement | From initiation of rising the breastbone and elbows until standing. |
| Duration (s) | Mean duration of the getting up movements. |
| Laterality – left side | The cow is lying on the left side |
| Percent | Percent times the cow is lying down on the left side out of all lying down sequences. |

| | |
|---|---|
| Changing side | The cow changes its lying side without standing up. |
| Number of cows | Has the cow been changing side; yes or no. |
| Lying down intentions and interruptions[4] | |
| Oscillating head movements | Oscillating movements before lying down is interpreted as lying down intentions[1]. |
| Bouts with previous oscillating movements (%) | Percent times out of all lying bouts were the cow performs oscillating movements before lying down: number of bouts with oscillating movement divided by the total number of bouts. |
| Latency time (s/bout) | Mean duration from the first oscillating head movement until the cow is lying down. When no oscillating movements are seen, the latency time is set to zero and still included in the calculation of the mean. |
| Total oscillating time (s/18h) | The summarized duration of oscillating movements per 18 h. |
| Total oscillating time per bout (s/bout) | Mean total oscillating time per lying bout. |
| Sniffing/oscillating | Sniffing/oscillating before lying is interpreted as lying down intentions[1]. |
| Total sniffing/oscillating time (s/18 h) | The summarized duration of sniffing and oscillating movements per 18 h. |
| Total sniffing/oscillating time per bouts (s/bout) | Mean total oscillating/sniffing time per lying bout. |

[4] Two variables regarding lying intentions have been calculated. One is oscillating head movements and the other one includes both oscillating head movements and sniffing movements.

When calculating the latency from the initial oscillating head movement and until lying, lying bouts with no previous oscillating head movements were given a latency of zero and included in the data set. The rationale behind including a latency time of zero in the calculation of the mean is that while oscillating movements may indicate that the cows is hesitant to lie down, a low degree of oscillating head movements, or none, may reflect a situation where the cow finds it easy to lie down. Lying laterally, lying down interruptions and changing side were turned into nominal data, due to the low frequency of these behaviors. Variables that were not normal distributed were either log (lying down and getting up movement, latency time, total sniffing/oscillating time and total sniffing/oscillating time per bout) or square root transformed (total oscillating time and total oscillating per bout).

*Statistical analysis*

All statistical analyses were performed using SAS software (version 9.4, SAS Institute Inc., Cary, NC). The variables total lying time, duration and frequency of lying bouts, duration of lying down and getting up movements, laterality, latency from the initial lying intentions until lying, total duration of oscillating head movements and sniffing/oscillating movements were analyzed by a mixed model (PROC MIXED Procedure). To account for the paired observations on each cow, cow was included as a random effect. The mixed model included the fixed effects of lying surface, lactation number, DIM and order of lying surface. Lying surface was classified as sand or rubber, lactation number as 1st, 2nd or later, DIM as early, late or dry and the order of lying surface was classified as SR (sand day 1 and rubber day 2) or RS (rubber day 1 and sand day 2). Order of lying surface were nested with pen number, to account for the fact that cows in pen I and II were always restricted to deep bedded sand on day 1, and cows in pen III and IV restricted to rubber mats on day 1. Two-way interactions between lying surface and the three other fixed effects were included. The same model was used to analyze all response variables, since model reduction did not improve the model fit monitored using Akaike's information criterion (AIC). After modeling each variable, the distribution of residuals of the model was checked graphically for the assumption of normality.

The variables lying laterally and lying down interruptions were transformed into nominal data and analyzed using McNemar's test (PROC FREQ Procedure).

Results from the mixed model are presented as least square means with standard errors and p-values. When back-transformed means are presented, the 95% confidence interval, also back-transformed, are reported. Statistical significant differences are reported when $p < 0.05$, and tendencies when $0.05 < p < 0.10$. The effects of fixed effect will only be reported when significant.

# Results

## Duration and frequency of lying

The effects of the lying surface on lying time and frequency of lying bouts during the 18 h observation period in the hospital pens are shown in Table 4. When the cows were kept on deep bedded sand, the lying time ($p<0.001$), the proportion of cows lying laterally ($p<0.006$, $\chi^2=8.33$, df=1), and the frequency of lying bouts ($p<0.004$) were higher than when kept on the rubber mats. On sand the lying time ranged from 28 min to 16 h. On rubber, the range was 6 min to 14 h. The mean duration of lying bouts did not differ between surfaces. The results of the statistical analysis showed that the order of the stay at the two lying surfaces nested with pen number affected the total lying time ($p=0.001$), showing that cows kept in pen III had a shorter total lying time compared to cows kept in pen IV ($p<0.002$) and pen II ($p<0.001$). In addition, cows kept in pen I had a lower total lying time than cows in pen II ($p<0.031$). The order of the stay at the two flooring types nested with pen number also had an effect on the frequency of lying bouts ($p=0.037$), where cows in pen III had a lower number of lying bouts compared to cows kept in pen I ($p=0.036$), II ($p=0,006$) and IV ($p=0,0763$).

Table 4. Least square means of lying time and frequency of lying for the 32 lame cows kept in individual hospital pens, where they were restricted to deep-bedded sand for 24 h and rubber mats for 24 h in a balanced order. The behavior of the cows were recorded for 18 h from 1200 h to 0600 h the following morning

| **Behavioral variables** | **Sand** | **Rubber** | **SE** | **F value** | **P value** |
|---|---|---|---|---|---|
| Total lying time | | | | | |
| Duration (h/18 h)[1] | 12.3 | 7.9 | 0.65 | 26.65 | < .001 |
| Lying laterally | | | | | |
| Number of cows[2] | 14 | 4 | - | - | = 0.006 |
| Lying bout | | | | | |
| Duration (min/bout)[1] | 74 | 70 | 6.58 | 0.27 | = 0.610 |
| Frequency (n/18 h)[1] | 11 | 7 | 0.94 | 9.93 | = 0.004 |

[1] Least square means from a mixed model accounting for cow as a random effect and order nested with pen, lactation number and DIM as fixed effects.

[2] Analyzed using McNemar's Test.

## Lying down movements

Table 5 shows the effects of the surface type on interruptions of lying behavior, the duration of lying-down and getting-up movements, as well as changes of side while lying. The proportion of cows showing interruptions of lying behavior did not differ between the two surfaces ($p=0.688$, $\chi^2=0.667$, df=1), as did not the number of cows changing side without standing. While kept on sand, the cows lay down and got up faster than on the rubber mats ($p=0.002$ and $p=0.047$, respectively). For the lying down movement, an effect of lactation number was found ($p=0.036$), showing that cows in their second lactation used longer time on the lying down movement than older cows.

Table 5. Least square means of lying down and getting up movements together with results regarding interrupted lying attempts and change of side for the 32 lame cows kept in individual hospital pens, where they were restricted to sand for 24 h and rubber mats for 24 h in a balanced order. The behavior of the cows was recorded for 18 h from 1200 h to 0600 h the following morning

| Behavioral variables | Sand | CI sand | Rubber | CI rubber | SE | F value | P value |
|---|---|---|---|---|---|---|---|
| Interruptions | | | | | | | |
| Number of cows[2] | 3 | - | 5 | - | - | - | 0.688 |
| Lying down movement | | | | | | | |
| Duration (s)[1,3] | 4.5 | 3.9 to 5.2 | 6.1 | 5.3 to 7.0 | - | 12.61 | 0.002 |
| Getting up movement | | | | | | | |
| Duration (s)[1,3] | 5.0 | 4.5 to 5.6 | 5.6 | 5.1 to 6.3 | - | 4.39 | 0.047 |
| Laterality | | | | | | | |
| Left side (%)[1] | 52.3 | - | 54.9 | - | 4.74 | 0.17 | 0.680 |
| Changing side | | | | | | | |
| Number of cows[4] | 1 | - | 1 | - | - | - | - |

[1] Least square means from mixed model accounting for cow as a random effect and order, nested with pen, lactation number and DIM as fixed effects.

[2] Analyzed using McNemar's Test.

[3] Back-transformed least squares means (95% CI) where natural log-transformation was applied to the variable.

[4] The behavior was only seen in one cow on each surface and no statistic was done on this variable.

## Lying intentions

In this study, oscillating head movements as well as a combination of sniffing behavior and oscillating head movements were included as lying intentions. Table 6 shows the values of these behavioral variables for the cows when kept on sand and the rubber mats. Five cows never showed oscillating head movements on sand and 3 cows did not show oscillating head movements at all. All 32 cows showed sniffing/oscillating movements. Overall, the proportion of lying bouts preceded by oscillating head movements did not differ between the lying surfaces (p=0.189). The latency from the initial oscillating movement until the cow was lying was lower when the cows were kept on deep-bedded sand compared to the rubber mats (p<0.001). No difference was found for the total duration of oscillating head movements per 18 h (p=0.429), but when calculated per lying bout, the duration of oscillating head movements tended to be shorter when the cows were kept on sand (p<0.098). For the combined variables, both the total duration as well as the duration per lying bout, were lower when the cows were kept on deep-bedded sand (p<0.001). The results of the statistical analysis showed that the two-way interaction between lactations number and lying surface affected the total duration of oscillating head movements (p=0.039), showing that cows kept in pen number III on the rubber mats were performing more oscillating head movements than when kept on sand in pen number III and also compared to cows kept in pen I on sand or rubber mats.

Table 6. Least square means of lying intentions from for the 32 lame cows kept in individual hospital pens, where they were restricted to sand for 24 h and rubber mats for 24 h in a balanced order. The behavior of the cows was recorded for 18 h from 1200 h to 0600 h the following morning

| **Behavioral variables** | **Sand** | **CI sand** | **Rubber** | **CI rubber** | **SE** | **F value** | **P value** |
|---|---|---|---|---|---|---|---|
| Lying down intentions | | | | | | | |
| Oscillating head movements | | | | | | | |
| Bouts with previous oscillating movements (%)[1] | 39.3 | - | 48.3 | - | 8.72 | 1.82 | 0.189 |
| Latency time (s)[1,2] | 10 | 2.6 to 41.7 | 151 | 37.2 to 602.6 | - | 21.69 | < .001 |
| Total oscillating time (s)[1,3] | 79 | 22.6 to 170.3 | 105 | 37.0 to 206.8 | - | 0.65 | 0.429 |
| Total oscillating time per bout (s)[1,3] | 9 | 2.3 to 19.9 | 18 | 7.4 to 32.2 | - | 2.96 | 0.098 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Sniffing/oscillating | | | | | | | |
| Total sniffing/oscillating time (s)[1,2] | 501 | 380.2 to 660.7 | 987 | 741.3 to 1349.0 | - | 16.85 | < .001 |
| Total sniffing/oscillating time per bout (s)[1,2] | 49.7 | 34.7 to 72.4 | 155.1 | 107.2 to 223.9 | - | 21.87 | < .001 |

[1] Least Square means from mixed model accounting for cow as a random effect and order, nested with pen, lactation number and DIM as fixed effects.

[2] Back-transformed least squares means (95% CI) where natural log-transformation was applied to the variable.

[3] Back-transformed least squares means (95% CI) where square root-transformation was applied to the variable.

# Discussion

This is one of the first studies examining the effect of lying surface on the lying behavior of lame cows, diagnosed with either white line disease or sole ulcers, kept in individual hospital pens. When kept on deep-bedded sand compared to rubber mats during the 18 h-recording period, the cows showed a higher total lying time, higher frequency of lying bouts, were lying more lateral, showed shorter duration of lying down and getting up movements as well as reduced occurrence of lying intention movements. These results suggest that lame dairy cows diagnosed with white line disease or sole ulcers have difficulty lying down and getting up on rubber mats compared to deep-bedded sand. Hence, deep bedding such as sand seems to be more favorable as a lying surface in hospital pens than harder types of flooring.

In the present experiment we found increased duration of lying for lame cows kept on deep bedded sand compared to rubber mats, which supports studies of lame dairy cows housed in free stalls (Cook et al., 2004; Gomez and Cook, 2010; Ito et al., 2010). Cows in pen III had a lower lying time and a lower number of lying bouts compared to cows in pen IV and II.

Three cows kept in pen III, for whom lying times below 2 h were recorded, may have caused this difference between the pens. The four pens were identical with the location in the barn being the only difference. Pen number III was located in the end of the barn furthest away from the gate, so the lower lying times are not a consequence of people entering the barn through the gate and the noises associated. We therefore believe that it is a coincidence that 3 cows with low lying times were placed in pen III.

Overall, the cows were lying for an average of 12.3 h on sand compared to 7.9 h on the rubber mats. Lying behavior is of high priority in cattle (Jensen et al., 2005), why it is recommended that dairy cows are allowed to lie down for at least 12 h per day and a lying time below 10 h has been shown to lead to physiological and behavioral stress responses (Munksgaard and Simonsen, 1996). Cows are expected to spend a greater proportion of time lying at night or during the very early morning hours (2300 h to 0500 h) (e.g. Mattachini et al., 2014) The 18-h observation period of the present experiment covered the period from 1200 h to 0600 h the following morning, thus including the period of the day were the cows were expected to lie the most. The present lying time show that the lame cows, when housed on deep-bedded sand, were able to obtain 12 h of rest per day, whereas it is unlikely that when housed on rubber, the cows would have been able to reach 12 h of lying per 24 h period, especially because cows are expected to spent a lower proportion of lying in the period from 0600 h to 1200 h compared to at night. A decrease in total lying time for lame cows has been associated with an increase in the duration of standing still, which is suggested to be an important risk behavior for the development of hoof lesions and may prolong the recovery after the lameness-inducing lesion (Cook and Nordlund, 2009). Time spent standing still has not been quantified in the present study, but the difference in lying time makes it likely that the cows might have shown an longer duration of standing still on the rubber mats compared to the deep-bedded sand. Hence, based on the earlier findings suggesting that rest is beneficial for lame cows (Cook and Nordlund, 2009) and considering their behavioral need for resting (Jensen et al., 2005), the present results suggest that housing lame cows on deep-bedded sand is advantageous compared to harder surfaces such as rubber mats.

The lying times observed on the two lying surface types in the present study varied considerably between the cows. Even though the cows were lying down for an average of 12.3 h on sand per 18 h, the minimum lying time observed was as low as 30 min. When housed on the rubber mats the lying time also varied a lot with a maximum individual lying time of almost 14 h and a minimum of only 6 min. Lying time in dairy cows is known to vary. Ito et al. (2009) reported individual means for lying time from 4.2 to 19.5 h per day and Leonard et al. (1996) reported that some heifers were lying only 5 h per day although the average lying time was 10 h in a group of 35 animals. The large variation shows the importance of not only using the mean lying time in a group of lame cows as an indicator of whether or not the cows fulfill their need for rest, but to be aware of the individual variation.

The lower total lying time observed when the cows were kept on the rubber mats was a result of a lower number of lying bouts compared to the period spent on sand, whereas no differences were found for the duration of single lying bouts. With the low number of lying bouts observed on the rubber mats it may be expected, that the lame cows would have increased the duration of each lying bout accordingly, in order to keep their normal lying time of 12 h or more per day (Munksgaard and Simonsen, 1996; Jensen et al., 2005). However, when kept on the rubber mats, the lame cows did not increase the duration of the lying bouts, which might be because rubber mats were not comfortable to lie on. This may be supported by the results showing fewer cows lying laterally when housed on the rubber mats (n=4) compared to the sand (n=14). Previous reports have shown comparable results regarding environmental effects on the frequency of lying laterally. Krohn and Munksgaard (1993) found a higher frequency of lateral lying on pasture, compared to open pens with deep bedding and tie stalls with either concrete or rubber surface. In a preference study, using the same cows as in this experiment, they showed that the lame cows prefer to lie down on deep-bedded sand compared to rubber mats when given the choice (Jensen et al., 2014), which might suggest that the cows finds it more comfortable to lie on deep bedded sand compared to rubber mats. Thus, we suggest, that the findings of cows not increasing the duration of individual lying bouts, as an attempt to keep their normal lying time, and that they avoided lying laterally may indicate that the deep-bedded sand was more comfortable to lie on than the rubber mats.

It has been suggested that it is easier for lame cows to get up and lie down on deep-bedded sand compared to a rubber mat (Cook and Nordlund, 2009), however this hypothesis has not been tested. The present findings of a lower number of lying bouts on the rubber mats compared to deep-bedded sand indicates, that the lame cows had difficulties – perhaps due to pain - when getting up and lying down on the rubber mats compared to the deep-bedded sand, as suggested by Cook and Nordlund (2009). These authors argued that sand allows the cows, and especially lame animals, to lie down and get up more easily compared to rubber mats, due to the cushion and traction provided by the deep sand. In the present study, we quantified the duration of the getting up and lying down sequences for the lame cows when kept on two different types of lying surfaces. The lame cows were shown to use longer time to lie down and get up when kept on rubber mats than the sand. In addition, cows in their second lactation used longer time on the lying down movement compared to older cows, where no difference between first lactation cows and older cows were observed. We would have expected younger, less heavy cows, to use less time on the lying down movements compared to older cows, but this study was not designed to investigate the effect of parity and although all cows entered the study with a gait score of 4, severity of the conditions, or number of affected hooves, were not balanced between parities. Measurements of the lying down and getting up movement can be used to evaluate cattle environments (Lidfors, 1989), with long durations being interpreted as the animals having difficulties lying down or getting up (Krohn and Munksgaard, 1993). An alternative sign of getting-up and lying down difficulty is the number of interrupted lying down sequences (Müller et al., 1989). In the present study no difference in the number of lying down interruptions was found. However, the longer durations of lying down and getting up movements, when the cows were kept on the rubber mats may suggest that the cows had difficulty lying down and getting up on the mats compared to the sand, which is supported by the lower frequency of lying bouts observed when cows were kept on the mats. Importantly, the relatively low duration of the getting-up (5.0 s and 5.6 s for sand and rubber respectively) and lying-down (4.5 s and 6.1 s for sand and rubber respectively) sequences observed in the present study suggest that the performance of these behavioral sequences were not problematic on either of the two lying surface types, when the cows were kept in spacious individual hospital pens. In the literature, duration of lying down movements on pasture and deep bedding (in loose housing) have been reported to be 7 and 8 seconds respectively, using a comparable ethogram to the one used in the present study (Krohn and Munksgaard, 1993). The slight shortening of the duration of these behavioral sequences found in the present experiment, might suggest that the cows were not having problems when lying down or getting up or that possible difficulties were not reflected in longer duration of these behavioral sequences. The comparison of getting up and lying down movements between studies can be difficult even though the same definition is used, because it can be challenging to be consistent in the recordings of these behaviors because they do not have a clear start and end point. This is also showed by the getting up variable having the lowest reliability of the ones tested in this experiment. One way to evaluate whether the lying down and getting up behavior were more difficult on rubber, was by including the latency and duration of lying intentions, expressed as oscillating head movements (Krohn and Munksgaard, 1993; Tucker and Weary, 2004; Niss et al., 2009). However, in the present study only approximately 40 % of the lying down sequences on sand and nearly 50 % of the sequences performed on the rubber mats, were preceded by this behavior, and in 3 cows this behavior were never seen. In the literature, there are several studies using the latency from the initial oscillating head movement after getting up and until the cow is lying down, as an indirect measure of the thwarting of lying motivation (Müller et al., 1989; Krohn and Munksgaard, 1993; Jensen, 1999). However, none of these report whether some cows did not perform this behavior, and how this was treated statistically. In the present analyses, lying bouts, where a cow did not performed any oscillating head movements before lying down, were given a latency of zero seconds, based on the expectancy that increased latency is an expression of thwarted lying motivation, and vice versa. The present finding of five cows, only performing the oscillating head movements when kept on rubber and not on sand, illustrates that this behavior belonged to the behavioral repertoire of these individuals, and that the lack of oscillating head movements must have been dependent on the environment. Hence, regarding the latency from oscillating head movements and until lying, we considered it misleading to analyze these cases as missing values.

The present study included another measure of lying motivation, the occurrence of the combination of oscillating and sniffing, which was probably less specific for lying motivation (sniffing has been interpreted as exploration (Munksgaard and Simonsen, 1996)) than the oscillating movements, but was performed by all the experimental cows and recorded before all lying down movements. The present hospital pens were novel to the cows and similarly the deep-bedded sand. In order to limit recordings of novelty induced sniffing, the video recordings for at least one hour after introduction of the cows to the pens were excluded from analyses. The fact that we found more oscillating/sniffing behavior when the cows were kept on rubber mats compared to the sand (sand was the novel substrate for the cows) indicates that the present findings were not expressions of exploration merely stimulated by novelty, but might be investigation of the surface to find a suitable lying location before lying down. Earlier reports have interpreted sniffing movements as lying intentions reflecting difficulties in lying down (Müller et al., 1989). These authors found that the frequency of sniffing as well as the latency from the initial sniffing within a bout of standing behavior and until the cow was lying down, was considerably higher in heifers housed on partially slatted floor compared to deep litter. In addition, they reported an increase in heart rate in tethered heifers kept on slatted floor, when the animals showed their first intention to lie down, in contrast to heifers housed on deep bedding, where the heart rate remained unchanged. Based on these results the authors concluded that the lying down movement on the slatted floor was particularly aversive to the cows (Müller et al., 1989). Thus, even though a proportion of sniffing movements might be expressions of other motivations such as exploration stimulated by novelty or searching for other biological relevant stimuli (such as food), we suggest that sniffing do reflect lying motivation (i.e. an intention to lie down).

The latency from sniffing until lying down was increased when the cows were kept on the rubber mats, suggesting that the cows were motivated to lie down but reluctant to do so, perhaps because the lying down movement is more difficult on the rubber mats compared to sand. This is supported by the lower frequency of lying bouts and the increased duration of the lying down movement on the rubber mats compared to sand. No difference between the lying surfaces were seen regarding the total duration of oscillating movements per 18 hours, which may be explained by the higher frequency of lying bouts on the sand. However, cows in pen III kept on the rubber mats were performing more oscillating head movements compared to when kept on the sand and compared to cows in pen I, which might suggest that cows kept on rubber in pen III are especially reluctant to lie down on rubber mats. Furthermore, the cows tended to perform more oscillating head movements per lying bout on the rubber mats compared to sand, suggesting that the lame cows, when kept on rubber mats, intended to lie down, but the lying intention was not followed by a lying bout, indicating that rubber mats restricted the lying down movements. The results regarding the total duration of oscillating/sniffing movements support this, as a higher duration of oscillating/sniffing movements was seen on the rubber mats (total duration as well as per bout).

This experiment has been carried out in spacious experimental individual hospital pens, but the knowledge can also be used in understanding the behavior of lame cows housed in other stall types. Studies have shown that sand as a bedding in free stalls compared to mattresses increases the lying time and that the prevalence of lame cows are lower in loose housing with free stalls bedded with sand compared to mattresses (Cook, 2003; Cook et al., 2004; Gomez and Cook, 2010; Ito et al., 2010). It has been suggested that the reason for shorter lying times in free stalls with mattresses and the higher prevalence of lame cows is due to the cows having more difficulties lying down and getting up when kept on rubber matrasses compared to deep bedded sand (Cook and Nordlund, 2009), this is supported by the results from this experiment.

# Conclusion

The results of the present experiment show that the lying behavior of lame cows kept in individual hospital pens is affected by the lying surface. When housed on sand lame dairy cows showed an increased total lying time, increased frequency of lying bouts, more lateral lying, decreased duration of lying-down and getting-up movements as well as a decreased duration of lying intention movements. These results suggest that lame dairy cows are more reluctant to lie down and get up on rubber compared to sand and that rubber is more unpleasant to lie on, which is reflected in the lower total lying time. Thus, we suggest that the provision of deep bedded lying surfaces such as deep-bedded sand to hospital pens may allow the animals to obtain their need for rest to a higher degree than when housed on harder surfaces such as rubber mats. These results have contribute with knowledge about how the lying surface affects the behavior of lame cows housed in hospital pens and can be used in the future when designing pens to lame cows.

# References

Anonymous. 2014. LBK nr 470 af 15/05/2014. Bekendtgørelse om lov om hold af malkekvæg og afkom af malkekvæg. *https://www.retsinformation.dk/Forms/r0710.aspx?id=162875%20*.

Barker, Z.E., K. a Leach, H.R. Whay, N.J. Bell, and D.C.J. Main. 2010. Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. *J. Dairy Sci.* 93:932–941.

Bruijnis, M.R.N., H. Hogeveen, and E.N. Stassen. 2010. Assessing economic consequences of foot disorders in dairy cattle using a dynamic stochastic simulation model. *J. Dairy Sci.* 93:2419–2432.

Clarkson, M.J., D.Y. Downham, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, R.D. Murray, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Incidence and prevalence of lameness in dairy cattle. *Vet. Rec.* 138:563–567.

Cook, N.B. 2003. Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface. *J. Am. Vet. Med. Assoc.* 223:1324–1328.

Cook, N.B., T.B. Bennett, and K. V. Nordlund. 2004. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. *J. Dairy Sci.* 87:2912–2922.

Cook, N.B., and K. V Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. *Vet. J.* 179:360–369.

Espejo, L.A., M.I. Endres, and J.A. Salfer. 2006. Prevalence of lameness in high-producing holstein cows housed in freestall barns in Minnesota. *J. Dairy Sci.* 89:3052–3058.

Fraser, D., D.M. Weary, E.A. Pajor, and B.N. Milligan. 1997. A scientufic conception of animal welfare that reflects ethical concerns. *Anim. Welf.* 6:187–205.

Galindo, F., and D.M. Broom. 2002. The effects of lameness on social and individual behavior of dairy cows. *Jounal Appl. Anim. Welf. Sci.* 5:193–201.

Gomez, A., and N.B. Cook. 2010. Time budgets of lactating dairy cattle in commercial freestall herds. *J. Dairy Sci.* 93:5772–5781.

Green, L.E., V.J. Hedges, Y.H. Schukken, R.W. Blowey, and A.J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. *J. Dairy Sci.* 85:2250–2256.

Haley, D.B., A.M. de Passillé, and J. Rushen. 2001. Assessing cow comfort: effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. *Appl. Anim. Behav. Sci.* 71:105–117.

Herlin, A.H. 1997. Comparison of lying area surfaces for dairy cows by preference, hygiene and lying down behaviour. *Swedish J. Agric. Res.* 27:189–196.

Hernandez-Mendo, O., M.A.G. von Keyserlingk, D.M. Veira, and D.M. Weary. 2007. Effects of pasture on lameness in dairy cows. *J. Dairy Sci.* 90:1209–1214.

Ito, K., M.A.G. von Keyserlingk, S.J. Leblanc, and D.M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. *J. Dairy Sci.* 93:3553–3560.

Ito, K., D.M. Weary, and M. a G. von Keyserlingk. 2009. Lying behavior: assessing within- and between-herd variation in free-stall-housed dairy cows. *J. Dairy Sci.* 92:4412–20.

Jensen, M.B. 1999. Adaptation to tethering in yearling dairy heifers assessed by the use of lying down behaviour. *Appl. Anim. Behav. Sci.* 62:115–123.

Jensen, M.B., M.S. Herskin, P.T. Thomsen, B. Forkman, and H. Houe. 2014. Lame cow's preferences for type of flooring and level of social contact in hospital pens. *In* Det rige liv - berigelse på tværs af dyrearter. Videncenter for Dyrevelfærd. 1–24.

Jensen, M.B., L.J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. *Appl. Anim. Behav. Sci.* 90:207–217.

Von Keyserlingk, M.G., J. Rushen, A.M. de Passillé, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle-key concepts and the role of science. *J. Dairy Sci.* 92:4101–4111.

Kiliç, N., A. Ceylan, S. ILker, and C. Gökbulut. 2007. Possible interaction between lameness, fertility, some minerals, and vitamin E in dairy cows. *Bull. Vet. Inst. Pulawy*. 51:425–429.

Krohn, C.C., and L. Munksgaard. 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. *Appl. Anim. Behav. Sci.* 37:1–16.

Leonard, F.C., J.M. O'Connell, and K.J. O'Farrell. 1996. Effect of overcrowding on claw health in first-calved friesian heifers. *Br. Vet. J.* 152:459–472.

Lidfors, L. 1989. The use of getting up and lying down movements in the evaluation of cattle environments. *Vet. Res. Commun.* 13:307–324.

Martin, P., and P. Bateson. 2007. Measuring behaviour: An introductory guide. Third edit. Cambridge University Press.

Mattachini, G., E. Riva, C. Bisaglia, J.C.A.M. Pompe, and G. Provolo. 2014. Methodology for quantifying the behavioral activity of dairy cows in freestall barns 1. 4899–4907.

Metz, J.H.M. 1985. The reaction of cows to a short-term deprivation of lying. *Appl. Anim. Behav. Sci.* 13:301–307.

Munksgaard, L., and H.B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. *J. Anim. Sci.* 74:769–778.

Müller, C., J. Ladewig, H.H. Thielscher, and D. Smidt. 1989. Behavior and heart rate of heifers housed in tether stanchions without straw. *Physiol. Behav.* 46:751–754.

Niss, D.B., M.S. Herskin, A.M. Danscher, and M.B. Thoefner. 2009. Short communication: rising and lying behavior of heifers before and after alimentary oligofructose overload. *J. Dairy Sci.* 92:617–620.

Plesch, G., N. Broerkens, S. Laister, C. Winckler, and U. Knierim. 2010. Reliability and feasibility of selected measures concerning resting behaviour for the on-farm welfare assessment in dairy cows. *Appl. Anim. Behav. Sci.* 126:19–26.

Rushen, J., E. Pombourcq, and A.M. de Passillé. 2007. Validation of two measures of lameness in dairy cows. *Appl. Anim. Behav. Sci.* 106:173–177.

Thomsen, P.T., L. Munksgaard, and J.T. Sørensen. 2012. Locomotion scores and lying behaviour are indicators of hoof lesions in dairy cows. *Vet. J.* 193:644–647.

Thomsen, P.T., L. Munksgaard, and F.A. Tøgersen. 2008. Evaluation of a lameness scoring system for dairy cows. *J. Dairy Sci.* 91:119–126.

Tucker, C.B., and D.M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? *J. Dairy Sci.* 87:2889–2895.

Walker, S.L., R.F. Smith, J.E. Routly, D.N. Jones, M.J. Morris, and H. Dobson. 2008. Lameness, activity time-budgets, and estrus expression in dairy cattle. *J. Dairy Sci.* 91:4552–4559.

Warnick, L.D., D. Janssen, C.L. Guard, and Y.T. Gröhn. 2001. The effect of lameness on milk production in dairy cows. *J. Dairy Sci.* 84:1988–1997.

Weary, D.M., L. Niel, F.C. Flower, and D. Fraser. 2006. Identifying and preventing pain in animals. *Appl. Anim. Behav. Sci.* 100:64–76.

# Expanded discussion

This thesis is a 45 ECTS master, which means that I have been responsible for collection and analysis of data, but not involved in the actual design of the study. The underlying video material originated from an experiment concerning preferences of lame dairy cows (Jensen et al., 2014). The experiment was planned before I started my master project. I knew from the beginning how much video material I could use, and have, supervised by M. B. Jensen and M. S. Herskin, been formulating aims and hypotheses for my master thesis. In this section, I will discuss the resultant experimental design and data analyses, the perspectives as well as my learning outcomes during the process of work with the master thesis.

As mentioned, the original experiment was designed to investigate preferences of lame dairy cows regarding lying surface and social contact when kept in individual hospital pens. As a part of this experiment, the experimental cows were restricted for 24 h on each type of surface to be compared (deep-bedded sand versus rubber mats), in order to standardize the level of experience with the two types before the period of free choice. My data constituted the two periods when the cows were restricted to the surface types. In the beginning of the process, where I had to decide on my aims and hypotheses, I was very optimistic regarding the amount of time needed to finalise this work. I wanted to investigate comfort behaviour, lying and social behaviour in order to examine effects of lying surface. Next, an ethogram was created and video analysis initiated. However, within days I realised that the initial ethogram included elements, the analysis of which was much too time consuming to be included in the temporal demands of a 45 ECTS project. Thus, I decided to focus on lying behaviour, since the lying behaviour of lame cows in free stalls is known to be affected by the lying surface and since lying behaviour has been suggested to affect the recovery of lame cows (Cook et al., 2004a; Gomez and Cook, 2010; Ito et al., 2010). Thus, right from the beginning I learned that each step within scientific work can be very time consuming and contains many different options of which you have to choose.

Due to the experiment not originally being designed to answer the question of my thesis, I had to make some compromises. For example, I had to exclude some variables, because I was not able to observe and record them systematically. Only having one video camera for each pen, covering the whole pen including both lying surfaces, was the reason for this. Hence, when the cows were kept on the flooring type closest to the camera, the view of the camera was focused on the back of the cow, whereas when the cows were kept on the part of the pen which was furthest away from the camera, the camera view was focused on the side of the cow. Thus, the angle of view was not the same for the two flooring types, which limited the range of behaviours that I was able to record. For example, I would like to have observed when the cows were bending their foreleg before lying down, but this was not always possible due to the cow standing in an angle to the camera, which made it impossible to see the front legs. Lying positions other than lying laterally were not possible to record, due to the position of the cows, sometimes lying in an angle to the camera which only made the back of the cow visible. Two cameras covering each pen would have improved the quality of the video recordings and allowed me to record a wider range of behaviours.

Regarding the interior design of the pens, improving the separation between the two lying surfaces may have optimised the design. Two simple gates consisting of a single rail were used to separate the two lying surfaces, making it easy to move the cows and remove the separation during the preference study. However, for my work, a solid gate dividing the two parts, where the cows were not able to see the other surface would have been advantageous. In addition, improved gates would have prevented one cow from trying to crawl under the gate in an attempt to reach the side of the pen with sand.

In addition, I would have preferred that the cows were kept on each surface for more than 24 h when considering the comparability of my result with other studies. As part of the standardisation of the preference study, the cows were kept for 24 h on each surface, but the exact timing of the moving of the cows varied between the blocks of the study. Hence, I had to exclude some of the video recordings, in order to make sure that I was observing all cows in the exact same time interval. I also wanted to exclude minimum the first hour they spend in the new pen, in order to minimise the occurrence of exploratory behaviour related to novelty captured on the videos. Thus, I observed the cows for 18 h from 1200 h to 0600 h the following morning. This means that all my results are based on 18 h of video recordings, which makes it hard to compare with results from previous studies, where periods of 24 h were used. If the cows had been kept for more than 24 h on each surface, I could still have excluded some of the recordings and gotten my results as measurements per 24 h. If the time the cows were restricted to each surface was expanded, we had to define some human endpoints where the cow should be taken out of the experiment in order to make sure that cows were not suffering, especially when restricted to rubber.

After having analysed the video recordings I had to decide how to analyse the data statistically. When deciding how to analyse data for scientific reports, it is important to take the study design and the primary aims of the study into account. In this case, the study was designed to investigate how the type of lying surface affects the behaviour of lame cows and not how factors such as lactation number, body condition score and DIM were affecting the behaviour. Due to the relatively small dataset it was not possible to estimate effects of factors other than lying surface. However, it is still important to be aware of these factors, which may explain some of the observed variation in behaviour, induced by the two types of lying surface. Hence, I included lactation number and DIM in the statistical model. Body condition score was not included due to the confounding with DIM and lactation number (Roche et al., 2007; Coffey et al., 2004). The order of the stay at the two lying surfaces, nested with pen number, was included as well, because I expected a difference in lying time between cows housed on sand on the initial day of the study versus cows housed on rubber on the initial day. These differences were expected, because cows restricted to the sand floor on the initial day might have lied less on the rubber mats on the day after. The opposite carry-over effect could also be expected with cows being extra motivated to lie on the deep-bedded sand after being restricted to the rubber mats, where the lying time was expected to be lower. However no carry-over effect was observed.

Thus, all possible two-way interactions including lying surface were included in the final statistical analyses. In the initial phases of analyses, three-way interactions were included as well, but their relatively low influence on the variance led to their exclusion from the final analyses. Since further reduction of the model, did not improve the fit of the model, the whole model was used for analyses of all response variables. This decision was based on an initial backwards-stepwise regression, removing fixed effects that were non-significant, using a significance level of 20%, 10% or 5 %, respectively. Removing fixed effects based on these exclusion criteria did not improve the model fit using Akaike's information criterion (AIC) why the whole model was used.
Forward-stepwise regression would have been another possible method of model creation. In this way, the initial analyses would only have included one or a few fixed effects, after which new effects should be added one by one until no improvement in the fitness of the model could be shown (Whittingham et al., 2014). The mixed model applied in the present statistical analyses was not appropriate for all the involved behavioural variables, due to low representation in the data. Even though some variables had a low representation in the

dataset, I did find these variables important and by including them in the results, I have made other people aware of the limited occurrence, which may be useful in future studies. For analysis, these variables were converted into nominal data and analysed non-parametrically by McNemar's test (Siegel and Castellan, 1988).

Regarding lying intentions, I decided to include the latency and the total duration of intentions movements in the results section. Initially, the frequency of lying intentions was included. However, this variable was excluded based on the overlap with the total duration of intention movements. The total duration of lying intentions is considered a more sensitive measure of thwarted lying motivation than the frequency of these movements, because the duration takes into account whether the behaviour was performed only very quickly or for longer periods. As discussed previously, the sniffing movements were seen in relation to lying behaviour and might be seen as the cows exploring the surface to find a suitable lying location, but sniffing movements might also be observed in relation to exploration of the new environment. It could have been interesting to examine whether the occurrence of sniffing movements was constant over the 18 h observed. Findings of lack of decrease in occurrence of the oscillation/sniffing would suggest that these behaviours did actually express lying motivation and not exploratory behaviour related to novelty. However, this was not included in the present work.

Importantly, I have learned during my work with statistical analysis of the present data, that there is not just one way, but several ways to perform statistical analysis each with pros and cons. Before I started the statistical analysis, I thought that there was only one right way to do it, and in the beginning I found it really frustrating that I was not able to find one such solution. During the process of writing the thesis, I have also learned how important it is to keep in mind what it is you want the readers to know and understand and to make the important points very clear. Especially concerning a research area with a lot of available literature, as is the case with bovine lying behaviour and lameness, the process becomes fragmented and looses focus if the goal of the work is not kept clear. In addition, I learned that the process of converting verbal explanations and hypotheses into scientific writing is not straightforward and takes practice.

To answer the question whether housing on sand compared to rubber improves the welfare of the lame cows further investigations are required. According to Frasers definition of animal welfare, the welfare of an animal may be based on three conceptions: The affective state of the animal, the functioning of the animal and the naturalness of the environment in which the animal is kept including the ability of the animal to perform natural behaviour (Fraser et al., 1997). The findings in the present study of increased duration of lying down and getting up movements and the increased duration of lying intention movements on rubber mats compared to deep-bedded sand may suggest that lame cows find it more painful to get up and lie down on rubber compared to sand. The lower lying time observed on rubber might also lead to behavioural and physiological stress reactions (Munksgaard and Simonsen, 1996). Based on these findings it can be suggested that keeping lame cows on sand improves the affective state of the animal. In addition, sand allows the lame cows to keep a daily lying time of 12 h or more, which suggests that they are able to keep their normal lying pattern to a higher degree than when kept on rubber. The low lying time on rubber might also affect the cow physically, since low lying time has been associated with a decrease in circulating levels of growth hormone (Munksgaard and Løvendahl, 1993), a short term increase in plasma cortisol levels (Fisher et al., 2002) and has furthermore been suggested to increase the incidence of lameness (Singh et al., 1994). Thus lame cows kept on deep-bedded sand may have an improved affective state, a better ability to perform natural behaviour and increased functioning and therefore also improved welfare.

During the work with lying behaviour in lame cows, some suggestions for future studies have arisen. My work has shown that the surface type of hospital pens does affect the lying behaviour of lame cows. When kept on sand floor, the cows were lying for longer, had a higher number of lying bouts, shorter duration of lying down and getting up movements and performed less lying intention behaviour before lying down as compared to the same animals kept on rubber mats. Relations between these differences in behaviour and recovery after lameness should be examined further. To the best of my knowledge it has not yet been investigated whether increased lying time is actually beneficial for cows, which are already lame. Based on the high prevalence of lameness and the effect on productivity and welfare it would be highly relevant to investigate this possible relations in order to understand how to improve recovery.

Furthermore, it has been suggested that the lying down and getting up movement is particularly difficult for lame cows (Cook and Nordlund, 2009). This suggestion may be examined by comparison of the duration of the getting up and lying down movements between non-lame and lame cows. Such results would, however, not clarify whether a possible increased duration of lying down and getting up movements is due to pain or caused by for example a stiff joint. In order to investigate whether pain is the reason for an increased duration of lying down and getting up movements, quantification of these behaviours before and after analgesic treatment might be advantageous.

In this experiment the effect of sand versus rubber mats as lying surface was investigated. The results suggest, as expected, that sand allowed lame cows to obtain their need for rest to a higher degree than the rubber mats. But this study has not examined how the lying surface is affecting others kinds of behaviour than lying behaviour. A study by (Jensen et al., 2014), using the same animals as in this present study, suggests that lame cows preferred to perform self-grooming while on rubber mats compared to deep-bedded sand, which leads to the question whether the type of flooring should be similar in the whole hospital pen or that different surfaces covering different parts of the pen will improve the ability of the lame cow to perform different behaviours. In order to answer this, further investigation is required, where the effect of different lying surfaces on different behaviours are investigated

In Denmark it is mandatory by law to house sick and injured cattle in hospital pens with soft and dry bedding. In newer buildings taken into use after 2012 the farmer must also be able to isolate sick and injured cows in individual hospital pens (Anonymous, 2014). The reasoning behind this legislation is that sick animals often seek isolation (Johnson, 2002) and thus that sick cows will be motivated to isolate themselves from the flock. Sickness behaviour is suggested to be an adaptive motivational state, initiated by the release of cytokines via the immune system, which leads to fever and changes in behaviour in order to maintain homeostasis (Johnson, 2002). The behavioural changes may be seen as: loss of appetite, increased in resting, a reduction in grooming behaviour and isolation seeking (Johnson, 2002; Broom, 2006; Almeida et al., 2008). The behavioural changes seen in lame cows in general (as described in the background) is for example an increased time spend lying (Chapinal et al., 2009; Ito et al., 2010; Thomsen et al., 2012), less time spend feeding (González et al., 2008; Gomez and Cook, 2010) and then lame cows are less active than non-lame cows (O'Callaghan et al., 2003; Walker et al., 2008). These changes in behaviour might be a consequence of the pain in the foot experienced by the lame cow and not a result of sickness behaviour. The lame cows in this experiment had non-infectious horn lesions and none of the cows had fever (<39.5 °C), which suggests that the behaviour observed in lame cows is not a consequence of sickness behaviour but a result of the pain in the affected hoof. Lame cows might therefore not benefit from being isolated in an individual hospital pen, which is supported by the a study investigating lame cows preferences for social contact (Jensen et al., 2014). This study showed that lame cows housed individually preferred to be near companion animals. It may therefore be suggested that shared hospital pens is a better way of housing lame dairy cows compared to individual hospital pens. In the future, investigating further whether lame cows should be housed individually or in groups would be relevant.

In this experiment a soft and deep-bedded lying surface (sand) was compared to a harder lying surface (rubber mats). I suggest investigating the effect of other types of lying surfaces on the behaviour of lame cows. Especially whether other types of deep bedding have the same positive effects as sand. Straw is a common used bedding and it would be relevant to investigate if a difference in the behaviour of lame cows is observed between a deep bed of sand and a deep bed of straw.

In this thesis I showed that lame cows, housed in individual hospital pens, kept on deep bedded sand may be better able to meet their need for rest than lame cows kept on rubber mats. This knowledge can be useful in the future when designing hospital pens for both lame and non-lame cows and also for further investigations of the handling of lame cows and how to design an environment that suits their needs as best as possible in order to increase the welfare.

# References

Almeida, P.E., P.S.D. Weber, J.L. Burton, and a J. Zanella. 2008. Depressed DHEA and increased sickness response behaviors in lame dairy cows with inflammatory foot lesions. *Domest. Anim. Endocrinol.* 34:89–99.

Amstel, S.R., and J.K. Shearer. 2006. Review of Pododermatitis Circumscripta (Ulceration of the Sole) in Dairy Cows. *J. Vet. Intern. Med.* 20:805–811.

Anonymous. 2014. LBK nr 470 af 15/05/2014. Bekendtgørelse om lov om hold af malkekvæg og afkom af malkekvæg. *https://www.retsinformation.dk/Forms/r0710.aspx?id=162875%20*.

Barker, Z.E., K. a Leach, H.R. Whay, N.J. Bell, and D.C.J. Main. 2010. Assessment of lameness prevalence and associated risk factors in dairy herds in England and Wales. *J. Dairy Sci.* 93:932–941.

Bell, N.J., D.C.J. Main, H.R. Whay, T.G. Knowles, M.J. Bell, and A.J.F. Webster. 2006. Herd health planning : farmers' perceptions in relation to lameness and mastitis. *Vet. Rec.* 159:699–705.

Bergsten, C., and A.H. Herlin. 1996. Sole haemorrhages and heel horn erosion in dairy cows: the influence of housing system on their prevalence and severity. *Acta Vet. Scand.* 37:395–408.

Blowey, R. 2005. Factors associated with lameness in dairy cattle. *In Pract.* 27:154–162.

Booth, C.J., L.D. Warnick, Y.T. Gröhn, D.O. Maizon, C.L. Guard, and D. Janssen. 2004. Effect of lameness on culling in dairy cows. *J. Dairy Sci.* 87:4115–4122.

Borderas, T.F., B. Pawluczuk, A.M. de Passillé, and J. Rushen. 2004. Claw hardness of dairy cows: relationship to water content and claw lesions. *J. Dairy Sci.* 87:2085–2093.

Broom, D.M. 1991. Animal welfare: concepts and measurement. *J. Anim. Sci.* 69:4167–4175.

Broom, D.M. 2006. Behaviour and welfare in relation to pathology. *Appl. Anim. Behav. Sci.* 97:73–83.

Capion, N., S.M. Thamsborg, and C. Enevoldsen. 2008. Prevalence of foot lesions in Danish Holstein cows. *Vet. Rec.* 163:80–86.

Chapinal, N., A.M. de Passillé, D.M. Weary, M.A.G. von Keyserlingk, and J. Rushen. 2009. Using gait score, walking speed, and lying behavior to detect hoof lesions in dairy cows. *J. Dairy Sci.* 92:4365–4374.

Clarkson, M.J., D.Y. Downham, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, R.D. Murray, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Incidence and prevalence of lameness in dairy cattle. *Vet. Rec.* 138:563–567.

Coffey, M.P., G. Simm, J.D. Oldham, W.G. Hill, and S. Brotherstone. 2004. Genotype and diet effects on energy balance in the first three lactations of dairy cows. *J. Dairy Sci.* 87:4318–4326.

Cook, N.B. 2003. Prevalence of lameness among dairy cattle in Wisconsin as a function of housing type and stall surface. *J. Am. Vet. Med. Assoc.* 223:1324–1328.

Cook, N.B. 2009. Free-stall comfort design for maximum cow. *WCDS Adv. Dairy Technol.* 21:255–268.

Cook, N.B., T.B. Bennett, and K. V. Nordlund. 2004a. Effect of free stall surface on daily activity patterns in dairy cows with relevance to lameness prevalence. *J. Dairy Sci.* 87:2912–2922.

Cook, N.B., and K. V Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. *Vet. J.* 179:360–369.

Cook, N.B., K.V. Nordlund, and G.R. Oetzel. 2004b. Environmental influences on claw horn lesions associated with laminitis and subacute ruminal acidosis in dairy cows. *J. Dairy Sci.* 87:36–46.

Duncan, I.J., and J.C. Petherick. 1991. The implications of cognitive processes for animal welfare. *J. Anim. Sci.* 69:5017–5022.

EFSA. 2009. Scientific report of EFSA prepared by the Animal Health and Animal Welfare Unit on the effects of farming systems on dairy cows welfare and disease. *Annex to EFSA J.* 1143:1–38.

Espejo, L.A., M.I. Endres, and J.A. Salfer. 2006. Prevalence of lameness in high-producing holstein cows housed in freestall barns in Minnesota. *J. Dairy Sci.* 89:3052–3058.

Ettema, J.F., and S. Østergaard. 2006. Economic decision making on prevention and control of clinical lameness in Danish dairy herds. *Livest. Sci.* 102:92–106.

Fisher, A.D., G.A. Verkerk, C.J. Morrow, and L.R. Matthews. 2002. The effects of feed restriction and lying deprivation on pituitary – adrenal axis regulation in lactating cows. *Livest. Prod. Sci.* 73:255–263.

Flower, F.C., and D.M. Weary. 2006. Effect of hoof pathologies on subjective assessments of dairy cow gait. *J. Anim. Sci.* 89:139–146.

Fraser, D., D.M. Weary, E.A. Pajor, and B.N. Milligan. 1997. A scientufic conception of animal welfare that reflects ethical concerns. *Anim. Welf.* 6:187–205.

Fregonesi, J.A., and J.D. Leaver. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. *Livest. Prod. Sci.* 68:205–216.

Galindo, F., and D.M. Broom. 2000. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. *Res. Vet. Sci.* 69:75–79.

Galindo, F., and D.M. Broom. 2002. The effects of lameness on social and individual behavior of dairy cows. *Jounal Appl. Anim. Welf. Sci.* 5:193–201.

Gomez, A., and N.B. Cook. 2010. Time budgets of lactating dairy cattle in commercial freestall herds. *J. Dairy Sci.* 93:5772–5781.

González, L.A., B.J. Tolkamp, M.P. Coffey, A. Ferret, and I. Kyriazakis. 2008. Changes in feeding behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *J. Dairy Sci.* 91:1017–1028.

Green, L.E., V.J. Hedges, Y.H. Schukken, R.W. Blowey, and A.J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. *J. Dairy Sci.* 85:2250–2256.

Haley, D.B., A.M. de Passillé, and J. Rushen. 2001. Assessing cow comfort: effects of two floor types and two tie stall designs on the behaviour of lactating dairy cows. *Appl. Anim. Behav. Sci.* 71:105–117.

Haley, D.B., J. Rushen, and A.M. de Passillé. 2000. Behavioural indicators of cow comfort: activity and resting behaviour of dairy cows in two types of housing. *Can. J. Anim. Sci.* 80:257–263.

Hedges, J., R.W. Blowey, A.J. Packington, C.J. O'Callaghan, and L.E. Green. 2001. A longitudinal field trial of the effect of biotin on lameness in dairy cows. *J. Dairy Sci.* 84:1969–1975.

Herlin, A.H. 1997. Comparison of lying area surfaces for dairy cows by preference, hygiene and lying down behaviour. *Swedish J. Agric. Res.* 27:189–196.

Ito, K., M.A.G. von Keyserlingk, S.J. Leblanc, and D.M. Weary. 2010. Lying behavior as an indicator of lameness in dairy cows. *J. Dairy Sci.* 93:3553–3560.

Jensen, M.B., M.S. Herskin, P.T. Thomsen, B. Forkman, and H. Houe. 2014. Lame cow's preferences for type of flooring and level of social contact in hospital pens. *In* Det rige liv - berigelse på tværs af dyrearter. Videncenter for Dyrevelfærd. 1–24.

Jensen, M.B., L.J. Pedersen, and L. Munksgaard. 2005. The effect of reward duration on demand functions for rest in dairy heifers and lying requirements as measured by demand functions. *Appl. Anim. Behav. Sci.* 90:207–217.

Johnson, R.. 2002. The concept of sickness behavior: a brief chronological account of four key discoveries. *Vet. Immunol. Immunopathol.* 87:443–450.

Von Keyserlingk, M.G., J. Rushen, A.M. de Passillé, and D.M. Weary. 2009. Invited review: The welfare of dairy cattle-key concepts and the role of science. *J. Dairy Sci.* 92:4101–11.

Krohn, C.C., and L. Munksgaard. 1993. Behaviour of dairy cows kept in extensive (loose housing/pasture) or intensive (tie stall) environments II. Lying and lying-down behaviour. *Appl. Anim. Behav. Sci.* 37:1–16.

Leonard, F.C., J.M. O'Connell, and K.J. O'Farrell. 1996. Effect of overcrowding on claw health in first-calved friesian heifers. *Br. Vet. J.* 152:459–472.

Lidfors, L. 1989. The use of getting up and lying down movements in the evaluation of cattle environments. *Vet. Res. Commun.* 13:307–324.

Metz, J.H.M. 1985. The reaction of cows to a short-term deprivation of lying. *Appl. Anim. Behav. Sci.* 13:301–307.

Munksgaard, L., and P. Løvendahl. 1993. Effects of social and physical stressors on growth hormone levels in dairy cows. *Can. J. Anim. Sci.* 853:847–853.

Munksgaard, L., and H.B. Simonsen. 1996. Behavioral and pituitary adrenal-axis responses of dairy cows to social isolation and deprivation of lying down. *J. Anim. Sci.* 74:769–778.

Murray, R.D., D.Y. Downham, M.J. Clarkson, W.B. Faull, J.W. Hughes, F.J. Manson, J.B. Merritt, W.B. Russell, J.E. Sutherst, and W.R. Ward. 1996. Epidemiology of lameness in dairy cattle: description and analysis of foot lesions. *Vet. Rec.* 138:586–591.

Müller, C., J. Ladewig, H.H. Thielscher, and D. Smidt. 1989. Behavior and heart rate of heifers housed in tether stanchions without straw. *Physiol. Behav.* 46:751–754.

Nocek, J.E. 1997. Bovine Acidosis : Implications on Laminitis. *J. Dairy Sci.* 80:1005–1028.

O'Callaghan, K. 2002. Lamneness and associated pain in cattle - challenging traditional perceptions. *In Pract.* 24:212–219.

O'Callaghan, K.A., P.J. Cripps, D.Y. Downham, and R.D. Murray. 2003. Subjective and objective assessment of pain and discomfort due to lameness in dairy cattle. *Anim. Welf.* 12:605–610.

Olechnowicz, J., and J.M. Jaskowski. 2011. Behaviour of lame cows : a review. *Vet. Med. (Praha).* 56:581–588.

Plesch, G., N. Broerkens, S. Laister, C. Winckler, and U. Knierim. 2010. Reliability and feasibility of selected measures concerning resting behaviour for the on-farm welfare assessment in dairy cows. *Appl. Anim. Behav. Sci.* 126:19–26.

Roche, J.R., D.P. Berry, J.M. Lee, K. a Macdonald, and R.C. Boston. 2007. Describing the body condition score change between successive calvings: a novel strategy generalizable to diverse cohorts. *J. Dairy Sci.* 90:4378–4396.

Rushen, J., E. Pombourcq, and A.M. de Passillé. 2007. Validation of two measures of lameness in dairy cows. *Appl. Anim. Behav. Sci.* 106:173–177.

Sanders, A.H., J.K. Shearer, and A. De Vries. 2009. Seasonal incidence of lameness and risk factors associated with thin soles, white line disease, ulcers, and sole punctures in dairy cattle. *J. Dairy Sci.* 92:3165–3174.

Siegel, S., and N.J. Castellan. 1988. Nonparametric statistics for the behavioral science. second. J. Anker, editor.

Singh, S.S., W.R. Ward, J.W. Hughes, K. Lautenbach, and R.D. Murray. 1994. Behaviour of dairy cows in a straw yard in relation to lameness. *Vet. Rec.* 135:251–253.

Tarlton, J.F., D.E. Holah, K.M. Evans, S. Jones, G.R. Pearson, and A.J.F. Webster. 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. *Vet. J.* 163:196–204.

The Alberta Dairy Hoof Health Project. Hoof Health. *http://www.hoofhealth.ca/Section4/article4_01.html*.

Thomsen, P.T., L. Munksgaard, and J.T. Sørensen. 2012. Locomotion scores and lying behaviour are indicators of hoof lesions in dairy cows. *Vet. J.* 193:644–647.

Thomsen, P.T., L. Munksgaard, and F.A. Tøgersen. 2008. Evaluation of a lameness scoring system for dairy cows. *J. Dairy Sci.* 91:119–126.

Tucker, C.B., and D.M. Weary. 2004. Bedding on geotextile mattresses: how much is needed to improve cow comfort? *J. Dairy Sci.* 87:2889–2895.

Tucker, C.B., D.M. Weary, and D. Fraser. 2003. Effects of three types of free-stall surfaces on preferences and stall usage by dairy cows. *J. Dairy Sci.* 86:521–529.

Walker, S.L., R.F. Smith, J.E. Routly, D.N. Jones, M.J. Morris, and H. Dobson. 2008. Lameness, activity time-budgets, and estrus expression in dairy cattle. *J. Dairy Sci.* 91:4552–4559.

Warnick, L.D., D. Janssen, C.L. Guard, and Y.T. Gröhn. 2001. The effect of lameness on milk production in dairy cows. *J. Dairy Sci.* 84:1988–1997.

Weary, D.M., L. Niel, F.C. Flower, and D. Fraser. 2006. Identifying and preventing pain in animals. *Appl. Anim. Behav. Sci.* 100:64–76.

Webster, A.J.F. 2001. Effects of housing and two forage diets on the development of claw horn lesions in dairy cows at first calving and first lactation. *Vet. J.* 162:56–65.

Wechsler, B., J. Schaub, K. Friedli, and R. Hauser. 2000. Behaviour and leg injuries in dairy cows kept in cubicle systems with straw bedding or soft lying mats. *Appl. Anim. Behav. Sci.* 69:189–197.

Welfare Quality® Consortium. 2009. Welfare quality assessment protocol for cattle.

Whay, H. 2002. Locomotion scoring and lameness detection in dairy cattle. *In Pract.* 444–449.

Whay, H.R., D.C.J. Main, L.E. Green, and A.J.F. Webster. 2003. Assessment of the welfare of dairy cattle using animal-based measurements: direct observations and investigation of farm records. *Vet. Rec.* 153:197–202.

Whittingham, M.J., P.A. Stephens, R.B. Bradbury, P. Robert, and R.P. Freckleton. 2014. Why do we still use stepwise modelling in ecology and behaviour ? 75:1182–1189.

Yunta, C., I. Guasch, and A. Bach. 2012. Short communication: lying behavior of lactating dairy cows is influenced by lameness especially around feeding time. *J. Dairy Sci.* 95:6546–6549.