The effects of stall surfaces and milk yield on the lying behavior of dairy cow

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ACADEMIC DISSERTATION

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Helsinki 2011
To cows, mothers of calves
Abstract

The importance of lying behavior to dairy cows and the feasible definition of lying has attracted many studies on the subject. Cattle show both behavioral and physiological stress responses when subjected to thwarting of their lying behavior. If cows are unable to lie down they later compensate for lost lying time when possible. Environmental factors such as housing and bedding systems have been noted to affect the time spent lying, but there is usually large variation in lying time between individuals. Internal factors such as the reproductive stage, age and health of cows affect their lying time and can cause variation. However, the effect of higher milk production on behavior has not previously been illuminated.

The objective of this study was to provide data applicable for the improvement of resting conditions of cows. The preference of stall surface material, differences in normal behavior per unit time and various health measures were observed. The aim was to evaluate lying behavior and cow comfort on different stall bedding materials. In addition, the effect of milk yield on behavior was examined in a tie stall experiment.

The preferences for surface materials were investigated in 5 experiments using 3 surface materials with bedding manipulations. According to the results, the cows preferred abundant straw bedding and soft rubber mats. However, they showed an aversion to sand bedding. Some individuals even refused to use stalls with sand when no organic bedding material was present. However, this study was unable to determine the reason for the avoidance, as neither the sand particle size nor thermal properties appeared critical. However, previous exposure to particular surface materials increased the preference for them.

The amount of straw bedding was found to be an important factor affecting the preferences for stalls, and the lying time in stalls increased when the flooring softness was improved by applying straw or by installing elastic mats. Despite sand being the least preferred flooring material in preference tests, the health of legs improved during exposure to sand-floored stalls. Moreover cows using sand were cleaner than those that used straw stalls. Thus, sand bedding entailed some health benefits despite the contradictory results of preference tests, which more strongly reflected the perceptions of individual animals.

Milk yield was observed to affect behavior by reducing the lying time, possibly due to factors other than longer duration of eating. High yielding cows seemed to intensify their lying bouts, as they were observed to lie with the neck muscles relaxed sooner after lying down than lower yielding cows.

In conclusion, cows were found to prefer softer stall surface materials and organic bedding material. In addition, the lying time was reduced by a high milk yield, although the lying time seemed to be important for resting. Cows might differ in the needs for their lying environment. The management of dairy cows should eliminate any unnecessary prevention of lying, as even in tie-stalls high yielding cows seem to be affected by time constraints. Adding fresh bedding material to stalls increases the comfort of any stall flooring material.
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List of original publications

This thesis is based on the following publications:

I Preferences of dairy cows kept in cold loose house for different kind of cubicle flooring. 

II Effects of Sand and Straw Bedding on the Lying Behavior, Cleanliness & Hoof and Hock Injuries of Dairy Cows. 

III Preferences of Dairy Cows for Three Stall Surface Materials with Small Amounts of Bedding. 

IV Milk yield affects time budget in dairy cows kept in tie-stalls. 
Norring, M., A. Valros & L. Munksgaard. (submitted J. Dairy Sci.)

The publications are referred to in the text by their Roman numerals.
1 Introduction

Resting is regarded as very important to all mammals (Tobler 1995). Cattle show both behavioral and physiological stress responses when subjected to thwarting of their lying behavior (Ladewig & Smidt 1989, Munksgaard & Simonsen 1995, Fisher et al. 2002), and chronic stress predisposes animals to diseases (Broom & Johnson 1993). Lying has a high priority and under time constraints cows allocate relatively more time to lying than to feeding (Munksgaard et al. 2005).

Animal welfare can be indirectly deduced by measuring the environment of the animals and judging its suitability. However, measures of an animal’s reactions provide more direct information on the perceived welfare. This study employed various methods to evaluate the reactions of cows to the production environment. The occurrence of and subtle changes in normal behavior over a fixed time unit were observed in addition to health measures. The time budget of dairy cows was examined to reveal changes associated with increasing milk yields. Finally, behavior tests were used to assess how cows perceived the resting places offered.

An uncomfortable resting environment can reduce the time spent lying and thus negatively affect welfare. However, comfort is not the only factor affecting the lying time. Although welfare status and lying time are undoubtedly associated, lying time can also be increased by health problems such as lameness. Therefore, conclusions about welfare based on lying time alone should be drawn carefully. Despite the caution towards the possibility to use lying time as an indicator of welfare, lying time on different surface materials and stall designs has been intensively studied (for example Herlin 1997, Tucker & Weary 2004, Tucker et al. 2004). Experimental settings provide control over factors potentially affecting lying behavior, and the effect of individual elements can be assessed independently. The studies in this thesis investigated the factors that influence the motivation of dairy cows to lie down. In the first studies, an increased lying time was considered as a sign of the level of comfort of the environment, whereas in the last experiment, lying behavior was treated as a reflection of differences in the physiological states of the cows. While the measures of lying behavior are difficult to interpret, the definition of lying is accurate and measurements can be objectively made (Plesch et al. 2010), which makes lying time an interesting subject of research.

When farmers build new cow sheds or renovate their stalls, they are interested in knowing the possible welfare and health consequences of alternative stall bedding materials. Sand stalls are considered to be hygienic (Zdanowicz et al. 2004) economical and comfortable (Cook 2003). Concrete stalls are traditional and relatively long lasting, while stalls with rubber mattresses are considered soft and comfortable (Herlin 1997), but expensive to renew regularly. Renewable organic bedding material is regarded animal friendly and hygienic.

The vision of this study was to provide dairy cows with better conditions for rest. This vision was approached by investigating the factors that affect lying behavior.
2 Aims and rationale

The aim of the studies reported in this thesis was to evaluate the lying behavior and comfort of cows on three stall bedding materials. The factors affecting the lying behavior of individual animals were also considered. The research investigated the aspects of cow lying behavior listed in Table 1.

Table 1  The research questions

<table>
<thead>
<tr>
<th>Question</th>
</tr>
</thead>
<tbody>
<tr>
<td>Which stall do cows prefer: straw-covered concrete, rubber mats or sand?</td>
</tr>
<tr>
<td>Is the sand particle size of importance for stall preference?</td>
</tr>
<tr>
<td>Does familiarity with the stall surface affect the lying time?</td>
</tr>
<tr>
<td>Does familiarity with the stall surface affect the preference?</td>
</tr>
<tr>
<td>Do cows choose a material that benefits their health?</td>
</tr>
<tr>
<td>Which stall material enhances cleanliness?</td>
</tr>
<tr>
<td>Is the amount of straw of importance for the preference?</td>
</tr>
<tr>
<td>Does milk yield affect the lying time?</td>
</tr>
<tr>
<td>Is latency to relax the neck after lying down affected by milk yield?</td>
</tr>
</tbody>
</table>

Variation in lying behavior was used as a measure of preference for certain stall surfaces or as a reflection of the motivation to lie down. The measurements aimed to determine how the animals perceived the situation. In addition, the effect of the environment on aspects of animal health was evaluated.
3 Lying behavior of dairy cows

The cow is considered as a crepuscular animal that rests in several periods during the day and night. The resting behavior is affected by many factors, both intrinsic and extrinsic (Figure 1). For example, the health and lactational stage of a cow can affect the daily lying time. In addition, stall size, crowding, and feeding routines can affect the lying time in free stalls.

According to Wierenga and Hopster (1990), the time spent lying by dairy cows is affected by the environment, management, and individual variation. Feeding can affect the lying time, which can be reduced, for example, by diets low in concentrate (Nielsen et al. 2000). Lying time can also increase as lactation proceeds (Chaplin et al. 2000a, Nielsen et al. 2000, Chaplin & Munksgaard 2001). However, the use of automatic milking systems can increase the time spent waiting (Ketelaar de Lauwere et al. 1996, Lexer et al. 2009), thus possibly reducing the time available for lying. Hänninen (2007) reviewed the factors affecting the lying behavior of calves, which are partly the same as those for adult cows. Temperature, age, space, the softness of the lying surface, feeding, social company, environmental changes, weaning, manipulations, and lightning all contribute to lying behavior of calves.

Knowledge of the natural lying behavior and habitat of cows could provide a basis for extrapolating the time needed for lying or the extent of lying motivation and preferred locations for lying. As the ancestors of cattle have become extinct, the only source of this type of information is from semi-feral cattle or those living in pasture conditions. Feral Chillingham cows have been observed to lie for only around 31% of the time (Hall 1989). Such a short lying time may reflect the avoidance of predation or foraging in a harsh environment. Modern breeds of high-producing dairy cows have been observed to use 40-50% of their time for lying in a naturalistic environment or on pasture (Webster 1993). Hassall et al. (1993) found that cows rested for about 8 hours per day on pasture, while Hernandez-Mendo et al. (2007) reported a lying time of 11 hours when cows were kept on pasture and 12 hours in free-stall housing. In a loose housing system where cows had access to pasture, they were observed to lie for 10 h per 24 h (Krohn & Munksgaard 1993).

In a tie stall, cows used 12.5 h per 24 h for lying (Krohn & Munksgaard 1993). Despite the lower lying times on pasture, cows prefer pasture for lying when given access to cubicles inside (Ketelaar-de Lauwere et al. 1999, Legrand et al. 2009). Contrasting results have also been recorded in a study by Olmos et al. (2009), in which cows spent more time lying on pasture (10 hours) than when housed in a cubicle system (9).
Figure 1  This cow is about to lie down. The status of the cow and several external factors affect the lying time.

3.1 The need for lying

Cows that are prevented from lying down show behavioral and physiological stress responses (Munksgaard & Simonsen 1995, Munksgaard & Simonsen 1996, Fisher et al. 2002, Cooper et al. 2007, Cooper et al. 2008). The need for resting has been shown by experiments where animals have been deprived of the opportunity for lying. During the recovery period after such deprivation, animals usually rest more (Ruckebusch 1974b, Metz 1985, Munksgaard et al. 2005, Cooper et al. 2007, Cooper et al. 2008). Thus the amount of rebound lying has been used as an estimate of the need for resting or tiredness. Ruckebusch (1974b) observed more sleeping after deprivation of lying, and a shorter sleep latency after lying down could therefore indicate a higher motivation to sleep.

The strongest evidence for the extent of the need for lying can be obtained by employing an operant conditioning technique. Jensen et al. (2005) reported in such
experiments that heifers were willing to work for access to lying. Heifers had an inelastic need for 12-13 hours of lying per day in a tethered situation.

While sleeping is one of the reasons why cows need to lie down, it may not be the only one. Cows divide their lying time between sleeping, ruminating and other behaviors. Lying behavior can be divided between active or inactive lying, ruminating or not ruminating, and if not ruminating, lying with the neck muscles relaxed or the head lifted up.

3.2 Internal factors affect lying behavior

There is profound individual variation among dairy cows in the time spent lying. The variation between animals is reported to be greater than that between farms (Ito et al. 2009). There are a number of possible reasons for the differences in lying times. For example, an older age can increase the lying time of dairy cows (Wierenga & Hopster 1990, Chaplin & Munksgaard 2001) although younger calves have been observed to rest more (Hänninen et al. 2003). Moreover cows later in lactation lie down for a longer time than those in the early stages of lactation (Chaplin et al. 2000a, Nielsen et al. 2000, Fregonesi & Leaver 2001, Olmos et al. 2009). Gestation possibly has similar effects.

Milk yield is suggested to be positively associated with the time spent lying and with lying comfort (Metcalf et al. 1992, Calamari et al. 2009). However, Österman & Redbo (2001) and Bewley et al. (2010) suggested that a higher milk yield would reduce the lying time. Nevertheless, the frequency of milking has not been found to affect the total lying time in experiments where cows have been milked either once or twice (Tucker et al. 2007), or twice or three times per day (Österman & Redbo 2001). The effect of milk yield on behavior has not been clarified. The more milk a cow produces the more feed it needs to consume (Dado & Allen 1994), and eating behavior takes place while standing. In addition, cows usually spend a longer time feeding when they receive more feed; although the time used for feeding is also dependent on the roughage and fiber content of the feed (Beauchemin et al. 1997). A high fiber content also increases time needed for ruminating (Beauchemin et al. 1997).

There are breed differences in bite rates (O’Driscoll et al. 2009) and in grazing behavior (Aharoni et al. 2009). However, an impact of breed or genetic composition on lying behavior has not been demonstrated (Nielsen et al. 2000). Genetic selection could have affected the lying behavior by drift or as a side effect of milk yield. The daily milk yield of cows has considerably increased during recent decades due to improved management, better nutrition and effective selection for higher production (Rauw et al. 1998, Hansen 2000).

The circadian rhythm can affect the timing of lying (Haley et al. 2000, Overton et al. 2002), although the innate rhythm is hard to detect and is inseparable from the management routines (DeVries & von Keyserlingk 2005, Fregonesi et al. 2007a, Tolkamp et al. 2010).

Short-term physiological alterations can affect the daily lying time. For example, estrus can reduce a cow’s time spent lying, and the deviation from normal pattern
continues for about 24 hours (Kiddy 1977, Hurnik & King 1987, Singh et al. 1994). Disease can change the lying behavior of a cow through the performance of sickness behavior (Weary et al. 2009), which includes increased resting, reduced activity and social isolation (Gregory 1998, Johnson 2002, Weary et al. 2009). In addition, lying time can change due to specific diseases. For example, milk fever can extend recumbency (Larsen et al. 2001). Moreover, claw diseases have been shown to increase the lying time and alter the movement and locomotory ability of diseased animals (Hassall et al. 1993, Singh et al. 1993b, Galindo & Broom 2002, Juarez et al. 2003, Walker et al. 2009).

3.3 Management factors affect lying behavior

Lying time can be affected by numerous factors related to the production environment and management. The effects of stall design on the behavior of dairy cows have been widely investigated. The stall dimensions, flooring and bedding material can affect the time cows spend lying. For example, limited stall dimensions can reduce the lying time (O’Connell et al. 1992, Haley et al. 2000, Tucker et al. 2004, Veissier et al. 2004, Fregonesi et al. 2009b). In preference tests, cows have been observed to select stalls with a softer flooring material for resting (Herlin 1997, Haley et al. 2001, Tucker & Weary 2004). The daily resting time has also been found to be reduced on hard flooring (Haley et al. 2000, Tucker & Weary 2004). Cows have been observed to lie down for longer bouts due to difficulties or pain inflicted by a hard floor or due to stall structures that restrict lying down and getting up (Lidfors 1989, Chaplin & Munksgaard 2001, Haley et al. 2000, Haley et al. 2001, Tucker & Weary 2004, Rushen et al. 2007). Abundant organic bedding material, such as straw or sawdust, is preferred by dairy cows as a lying surface compared to scarce or no bedding (Tucker et al. 2003, Tucker & Weary 2004, Tucker et al. 2009). Dry bedding has been shown to be preferred compared to wet bedding (Fregonesi et al. 2007b) and an insufficient amount of sand in sand stalls has been found to reduce the use of the stalls (Drissler et al. 2005). Overcrowding can reduce the lying time of the entire herd (Leonard et al. 1996, Wierenga & Hopster 1990, Fregonesi et al. 2007a, Hill et al. 2009) or the effect can be restricted to individuals lowest in the dominance order (Wiegenga & Hopster 1990). Furthermore, heat stress can cause reductions in lying behavior (Cook et al. 2007). In addition, the timing of feed delivery affects the distribution of lying during the day (DeVries & von Keyserlingk 2005).

3.4 Methods to evaluate stall comfort

Preference tests can be used to evaluate the relative preference between alternative stall surface materials. Preference tests and motivation tests are regarded as powerful tools for the assessment of animal welfare and feelings (Kirkden & Pajor 2006). The interpretation of preference test results is enhanced if the choices do not differ in many aspects. Several factors have been acknowledged to have confounding effects in the preference tests, such
as the prior experience and motivation of the animal, and the thermal conditions during the test (Duncan 1992). The short-term preference may conflict with the long-term preference or even with welfare (Duncan 1992). In addition, motivation tests can be used to answer questions about whether the motivation is altered by changes in the internal environment (Kirkden & Pajor 2006). Grandin et al. (1994) found cattle to be reluctant to change their choices in a Y-maze test. Preference tests have also been widely employed to examine the environmental conditions and stall surface materials preferred by dairy cows (e.g. Tucker et al. 2003, Tucker & Weary 2004, Tucker et al. 2004).

The total lying time over 24 hours is a widely used and easily compared measure (e.g. Tucker et al. 2003, Cook et al. 2008, Tucker at al. 2009). However, many factors can affect this measure. The resting behavior of dairy cow has been used as an indicator of welfare and cow comfort. Haley et al. (2000) found that cows rested more in a comfortable lying place than an uncomfortable one. Cows rested 4 h more in a soft-floored pen than in a hard-floored tie stall. The amount of time allocated, for example, to feeding, ruminating or lying (Phillips & Leaver 1986, Hasegawa et al. 1993, Fregonesi & Leaver 2001) can be used in discussion on the needs of the animals. The behavioral priorities can be evaluated in experiments where the time available for the animals to perform certain behaviors is limited (Munksgaard et al. 2005, Cooper et al. 2007).

Sleep consists of REM and non-REM stages. However, EEG activity needs to be measured to confirm an animal’s sleeping status. Sleeping in cattle has been described using various ethogram criteria (Ruckebusch 1974a, Hänninen et al. 2008). Behavioral signs of REM sleep in cows or calves are most often described as lying with the neck muscles relaxed (Ruckebusch 1974a, Ruckebusch et al. 1974, Hänninen et al. 2008). Ruckebusch (1975) observed no rumination during REM sleep, and estimated the total time spent sleeping to be around 4 hours per day.

Lameness and skin lesions are common causes of health problems. The stall surface material and restrictive stall dimensions are known to cause skin lesions on hocks, knees and legs (Rushen et al. 2007, Weary & Taszkun 2000, Vokey et al. 2001, Veissier at al. 2004, Fulwider et al. 2007). Lameness is usually a painful condition (Flower et al. 2008), and the same applies to skin lesions, as animals avoid the provocation of a sensitive spot. However, the etiology of hoof disorders is not well understood, although known risk factors include feeding, the production level and age (Onyiro et al. 2008, Cook & Nordlund 2009). Environmental risk factors for lameness include a wet floor surface, infectious diseases, the stall surface and housing type (Leonard et al. 1994, Cook et al. 2004, Borderas et al. 2004). Hoof disorders and lameness are generally alleviated by pasture conditions as compared to cubicle housing (Olmos et al. 2009).

Cows like to keep their fur groomed and severe dirtiness can cause skin irritation. In addition, cow cleanliness is suggested to affect udder health (Munoz et al. 2008, Schreiner & Ruegg 2003). Even a small amount of dirt on the udder is considered to constitute a risk for udder health (Reneau et al. 2005). Cow cleanliness can reflect the stall cleanliness (Fregonesi et al. 2009a). Differences between bedding materials have been found in their ability to sustain the growth of mastitis-causing pathogens (Hogan et al. 1998), and the bacteria on bedding have also been found to contaminate the teat skin (Zdanowicz et al. 2004).
4 Materials and Methods

The research questions (Table 1) were addressed in a series of experiments (Table 2).

<table>
<thead>
<tr>
<th>Table 2</th>
<th>The main experimental set-ups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>Preference for three materials was tested in the winter and summer</td>
</tr>
<tr>
<td>Experiment 2</td>
<td>Preference for stalls containing either rough sand or fine sand was tested</td>
</tr>
<tr>
<td>Experiment 3</td>
<td>Cows were housed in stalls with either sand or deep straw bedding for at least 21 weeks</td>
</tr>
<tr>
<td>Experiment 4</td>
<td>Preference for sand or straw stalls was tested after exposure to one or the other for at least 21 wk</td>
</tr>
<tr>
<td>Experiment 5</td>
<td>Preference for three materials with a small amount of bedding was tested</td>
</tr>
<tr>
<td>Experiment 6</td>
<td>The behavior of cows that varied in their milk yield was analysed</td>
</tr>
</tbody>
</table>

4.1 Animals and housing

The experiments were conducted between 1997 and 2004 in two different barns (Table 3).

<table>
<thead>
<tr>
<th>Table 3</th>
<th>The lying behavior of cows was investigated in 6 experiments. Experiment 6 was conducted in a tie stall while all other experiments were conducted in a loose house.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment</td>
<td>Year</td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
</tr>
<tr>
<td>1</td>
<td>1997-1998</td>
</tr>
<tr>
<td>2</td>
<td>1998</td>
</tr>
<tr>
<td>3</td>
<td>2000-2002</td>
</tr>
<tr>
<td>4</td>
<td>2001-2002</td>
</tr>
<tr>
<td>5</td>
<td>1999</td>
</tr>
<tr>
<td>6</td>
<td>2004</td>
</tr>
</tbody>
</table>

Cows

In total, 188 Friesian or Ayrshire dairy cows were included in the experiments (Table 4). Some of the cows were included in more than one experiment. For example, in Experiment 2, we used the cows that had been included in Experiment 1.
Table 4  
Numbers and characteristics of the cows used in the experiments. The milk yield of multiparous cows is expressed as kg milk during the previous lactation, while the yield of primiparous cows was measured before the beginning of the experiments. The primiparous cows were divided evenly between the groups. The cows entered Experiment 4 immediately following Experiment 3. (DIM = days in milk at the beginning of the experiment)

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
<th>Exp. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of cows</td>
<td>44</td>
<td>18</td>
<td>52</td>
<td>27</td>
<td>18</td>
</tr>
<tr>
<td>Primiparous cows</td>
<td>15</td>
<td>7</td>
<td>17</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>Multiparous cows</td>
<td>29</td>
<td>11</td>
<td>35</td>
<td>20</td>
<td>18</td>
</tr>
<tr>
<td>Milk yield of primiparous (kg/d)</td>
<td>26 ± 4</td>
<td>18 ± 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk yield of multiparous (kg)</td>
<td>7858 ± 1072</td>
<td>7470 ± 1183</td>
<td>7855 ± 1661</td>
<td>6874 ± 1193</td>
<td></td>
</tr>
<tr>
<td>Parity range of multiparous cows</td>
<td>2-5</td>
<td>2-5</td>
<td>2-7</td>
<td>2-7</td>
<td>2-7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>614 ± 72</td>
<td>571 ± 61</td>
<td>663 ± 81</td>
<td>615 ± 77</td>
<td>617 ± 65</td>
</tr>
<tr>
<td>DIM</td>
<td>116 ± 41</td>
<td>50 ± 23</td>
<td>30-195</td>
<td>58 ± 5</td>
<td></td>
</tr>
</tbody>
</table>

Table 5  
Description of the temperature and housing during the experiments

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
<th>Exp. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature °C</td>
<td>0 ± 5 c</td>
<td>2 ± 5 f</td>
<td>6 ± 9 f</td>
<td>4 ± 8 g</td>
<td>-20 to +8</td>
</tr>
<tr>
<td>Housing</td>
<td>Loose house</td>
<td>Loose house</td>
<td>Loose house</td>
<td>Loose house</td>
<td>Loose house</td>
</tr>
</tbody>
</table>

Farms

The experiments were conducted at two University of Helsinki farms, both situated in Southern Finland (Table 5). Experiments 1-5 (I-III) were conducted in Suitia barn which was an unheated, uninsulated cold loose house with cubicles (Figure 2). Experiment 6 was conducted in an insulated tie-stall barn in Helsinki (IV).
During the experiments the cows were fed grass silage *ad libitum*. Concentrates were provided by automatic feeders (in Exp. 1-5). In Experiment 6, concentrates were delivered by hand 6 times per day. The amount of concentrates fed varied according to the milk production of the cow. Cows were milked twice a day in a milking parlour or in their tie-stalls.

**Stalls**

The soft rubber mats (Cloud 9) used in the experiments had pegs of different lengths underneath to increase the softness (Table 6). Bedding was added so that the stall floor was covered with a thin layer of straw that barely covered the surface when spread evenly.

Sand stalls were constructed by adding a layer of about 20 cm rough or fine sand onto a sand base. The rough sand had a particle diameter of 2–3 mm and the fine sand a particle diameter of 0.1–0.6 mm. New sand was added to stalls when needed to keep the base constant. Extra cut barley straw bedding was added every other day and soiled straw and sand was removed when the stalls were cleaned.

**Table 6**  
*Summary of the stall surface materials used in the experiments.*

<table>
<thead>
<tr>
<th>Material of surface</th>
<th>Quality of material</th>
<th>Bedding</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
<th>Exp. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concrete</td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubber mat</td>
<td>Soft</td>
<td>covered with a thin layer of straw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Hard</td>
<td>sawdust bedding (tie stall)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Sand</td>
<td>Rough</td>
<td>no bedding</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>no bedding</td>
<td></td>
<td></td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fine</td>
<td>covered with a thin layer of straw</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>
The same stalls were used in experiments 1-5; the stall length was 248 cm with 204 cm available for lying and the rest consisting of head space. The stall width was 120 cm. Neck rails were positioned 163 cm from back part of the stalls at a height of 110 cm. The stalls had a 29-cm curb. In experiment 6, cows were kept in tie stalls (170 x 121 cm) with hard rubber mats (2 cm thick, DeLaval) bedded with sawdust.

4.2 Measurements

Video recording techniques

We used time lapse video recording with a 24-h time mode and cameras with a 3 to 8 mm wide-angle lens, and a 1/3 inch CCD matrix. Pictures from all cameras were fed into a single video recorder using a multiplexer (Robot). In the loose house studies the cameras were situated on average 7.4 m from the nearest stall. Cameras were placed so as to be able to film one whole row of stalls (Picture 1). To be able to identify the cows in the loose house, animals with very similar color patterns were marked using hair coloring. In the tie-stall barn a camera was placed in front of each stall and enough additional cameras were installed to be able to see the movement of cows' jaws in all possible positions.

Picture 1  Lying behavior was video recorded; the picture from several cameras could be monitored simultaneously.

Preference tests

Stall preference was tested by allowing cows to choose between two types of bedding in a test room. The cows indicated their preference by lying on one material. The lying behavior was observed 24 h a day during several days (Table 7). By using group means as a measure of preference, the animals could be kept in groups.
Preference tests were carried out after observations of lying time when cows could not choose between different materials. To ensure that tested cows had experience of both materials, the cows were exposed to both of them before the preference tests (no-choice phase). At the beginning of Experiment 1, the first groups tested were not subjected to a no-choice phase. During preference tests, behavior was observed using scan sampling (Noldus, Observer) at 9-minute intervals.

Table 7  Number of observation days for 24-h behavior (no-choice phase) and preference testing. The values are days unless otherwise indicated.

<table>
<thead>
<tr>
<th>Lying behavior per 24 h</th>
<th>Exp. 1</th>
<th>Exp. 2</th>
<th>Exp. 3</th>
<th>Exp. 4</th>
<th>Exp. 5</th>
<th>Exp. 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of days observed</td>
<td>2</td>
<td>2</td>
<td>11-18</td>
<td>-</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Average interval between observations</td>
<td>2</td>
<td>2</td>
<td>1-2 wk</td>
<td>-</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Duration of exposure per material</td>
<td>3</td>
<td>5</td>
<td>21-28 wk</td>
<td>-</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

Preference test

| Number of days observed | 6-8   | 4      | -      | 3      | 5      | -      |
| Average interval between observations | 3.2<sup>d</sup> | 2      | -      | 2      | 2      | -      |
| Duration of preference testing | 3-4 wk | 7      | -      | 5      | 10     | -      |
| Number of groups | 4 groups | 6 groups | 6 groups | 10 groups | 6 groups | g |
| Number of animals in group | 5-6<sup>e</sup> | 3      | 5-12<sup>f</sup> | 2-3    | 3      | -      |

<sup>c</sup> Two days for the adaptation week
<sup>d</sup> Range 1-4
<sup>e</sup> In winter
<sup>f</sup> 12, 9 or 5, depending on the year
<sup>g</sup> Tie stall

24-h lying time and time budget

Lying behavior was observed during periods when cows had no choice of stall material. The behavior was analysed using continuous observation (Table 8). The total duration of lying per 24 h and the frequency and duration of lying bouts were recorded. During experiment 6, the head and jaw movements in relation to feeding, rumination and resting behaviors were also recorded to construct a time budget.

Through the use of behavioral observations, the stall material preferences could be elucidated, but exposure to the preferred material could also provide advantages for cow health.
Table 8  

*Ethogram of the behaviors observed during the experiments and the definitions of observations*

<table>
<thead>
<tr>
<th>Posture</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>Rump on the floor</td>
</tr>
<tr>
<td>Standing</td>
<td>Standing on four feet</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feeding</td>
<td>Taking silage into the mouth and jaw moving, or muzzle in contact with silage and moving</td>
</tr>
<tr>
<td>Ruminating</td>
<td>Jaw moving, not taking silage into the mouth</td>
</tr>
<tr>
<td>Inactive without ruminating</td>
<td>Jaw not moving, head not moving, head up</td>
</tr>
<tr>
<td>Lying with the neck relaxed</td>
<td>Jaw not moving, neck relaxed and head in contact with the feeding table in front of the cow, or reversed along her body</td>
</tr>
<tr>
<td>Other activities</td>
<td>All other behaviors of the head not mentioned above, for example head moving actively, drinking, feeding on concentrate, licking, social behavior, leaning the muzzle towards equipment</td>
</tr>
</tbody>
</table>

**Leg health**

To compare leg health on different bedding materials and to relate these findings to cow behavior, leg health was recorded. In this study, leg health on sand and straw bedding was compared. Injuries to the legs and hoof health were scored once at the beginning and once at the end of Experiment 3. Swelling of the front knees was recorded by measuring the circumference of the knees using a tape measure. Lesions of back leg hocks were measured for size (Picture 2) and the severity of the lesions was scored using a 6-point scale (0 = no lesion; 5 = open cuts). The hoof health of cows was evaluated during hoof trimming. The presence or absence of claw diseases, i.e. the occurrence of sole hemorrhages, laminitis, heel horn erosion, white line disease, sole ulcer, corkscrew claw, and other rare cases, was scored separately for both claws on each leg. The results were compiled by giving 1 point for each claw half that had any of these claw diseases.
Cleanliness

The cleanliness of the cows was recorded to determine which material kept them cleaner. As cow cleanliness can be a reflection of stall cleanliness, the cleanliness of the stalls was also evaluated.

The cleanliness of the cows was evaluated once weekly during the last 7 weeks of Experiment 3 using a measuring system adapted from Haley et al. (2000). The materials compared were sand and straw bedding. The teats, other parts of the udder, belly, sides of the belly, and legs (lower, mid, and upper leg, on left and right rear legs) were evaluated separately, assigning 1 point for each area if any dirt or manure was visible, giving a maximum possible value for each cow of 10 for each evaluation. The average score for each cow was calculated over the 7 weeks.

The cleanliness of the stalls was evaluated using a 2-point scale twice a day, before the stalls were cleaned 2 d/wk during the last 4 weeks of Experiment 3. The values were summed to give a maximum possible value for each stall of 16.

Milk yield

Milk yield was measured to investigate or control for its effect on lying behavior. Experiment 6 focused on the amount of milk as a factor affecting the time budget of cows that were in the same stage of lactation (Table 4). In Experiment 6, the amount of milk was measured at every milking, and the average daily milk yield or milk yield at one milking was used in the analysis. The milk yield was balanced between the groups of animals in Experiments 1-5 (I-III).

Refinement of experimental procedures

The experiments were approved by the Animal Ethical Committee of the University of Helsinki, and they were conducted in the normal production environment in a manner that avoided any additional discomfort.
4.3 Statistical methods

Mixed models analysis was used with individual cows or cow groups as the statistical unit. When the structure of the data did not allow this type of analysis, Mann-Whitney and Wilcoxon tests were instead used.

Unless otherwise indicated, the results are presented as means ± SE. *P*-values less than 0.05 were considered significant. In Experiments 1 and 2, the statistical program SAS was used, whereas the rest of the analysis was conducted using SPSS (versions 12.0, 13.0 and 15.0 SPSS Inc., Chicago, Illinois).

During the no-choice periods, differences between materials in the total lying time per day, and in the duration and total number of lying bouts were tested with mixed models. In Experiment 1, the flooring material was used as a fixed and the cow as a random effect. In Experiment 3, the model included the material, year, and parity as fixed effects. In Experiment 5, average values of groups and days were analysed, with the stall surface material as a fixed measure, the period as a repeated measure and the group as a random effect.

In Experiment 6, the duration of the behaviors over 24 h and the percentage of behaviors per 4 h were analyzed using milk yield and parity as factors. The day was inserted as a repeated effect and cow was the subject. In addition, the average latency for each cow to the initiation of different behaviors after a commencement of a lying bout was analyzed.

In Experiment 1, the flooring material and day were used as factors in the model to test the preference. Differences were evaluated separately for each group. In Experiments 4 and 5, the average number of lying observations per cow group was used in the analysis and the Wilcoxon signed ranks test was applied.

To test the effect of hock lesion sizes, the day, leg and material were used as fixed factors in mixed models analysis, while Mann-Whitney U-tests were used to test the differences between materials in the dirtiness of stalls or cows and the severity of hock and hoof lesions.
5 Results

5.1 Lying time on different materials

During the no-choice periods in Experiment 1, when the cows had access to only one stall surface material, the lying time was shorter on sand than on straw or rubber mats in both winter and summer (Figure 3). There were no differences in the mean duration of lying bouts per day. However, there were fewer lying bouts per day on sand than on other materials (Table 9).

In Experiment 2, there were no differences between the two types of sand in total lying time, or the duration or frequency of lying bouts (Figure 3, Table 9).

Figure 3  Total lying time per day in the same cow house during Experiments 1, 2, 3 and 5. There were several differences between the experiments, for example in the amount of bedding and the duration of the experiment. * indicates a significant difference within the experiment (P < 0.05).
In Experiment 3, there was an interactive effect of day and bedding material on the total daily duration of resting during the adaptation period \( (P < 0.001) \). On day 1, the total duration of resting was lower for cows on sand than on straw. As the experimental period progressed, there was an increase in the total duration of resting for cows on sand, but no increase for cows on straw.

During the remaining observation period of 20 to 27 weeks, the daily duration of lying was longer for cows on straw than those on sand (Figure 3). This appeared as a result of a combination of a higher frequency of lying bouts and an increase in the duration because neither one alone differed between the materials (Table 9).

**Table 9**

The duration and total number of lying bouts during the experiments. In Experiment 2 the duration of lying bouts was 83 minutes ±6 on fine sand and 79 minutes ±5 on rough sand. The average total number of lying bouts on fine sand was 6.1 ±1.1 and on rough sand 6.5 ±1.2. Letters indicate a significant difference within rows.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Exp.</th>
<th>Straw</th>
<th>Rubber mat</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of lying bouts</td>
<td>1 Winter</td>
<td>65 ± 2.8</td>
<td>70 ± 4.5</td>
<td>67 ± 3.6</td>
</tr>
<tr>
<td>(min)</td>
<td>1 Summer</td>
<td>67 ± 4.2</td>
<td>73 ± 7.0</td>
<td>93 ± 16.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>76 ± 3.2</td>
<td>not included</td>
<td>72 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>76 ± 4.2</td>
<td>71 ± 7.0</td>
<td>71 ± 3.3</td>
</tr>
<tr>
<td>Total number of lying</td>
<td>1 Winter</td>
<td>11.9 ± 0.7(^a)</td>
<td>10.7 ± 0.7(^a)</td>
<td>6.8 ± 1.2(^b)</td>
</tr>
<tr>
<td>bouts</td>
<td>1 Summer</td>
<td>9.8 ± 0.8(^a)</td>
<td>9.3 ± 0.8(^a)</td>
<td>0.7 ± 0.4(^b)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.3 ± 0.5</td>
<td>not included</td>
<td>10.1 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>9.9 ± 0.5</td>
<td>11.1 ± 0.5</td>
<td>10.6 ± 0.5</td>
</tr>
</tbody>
</table>

In Experiment 5, the total duration of lying down was higher on rubber mats compared to concrete or sand (Figure 3). However, there were no effects on the frequency of bouts of lying down, or the mean duration of lying bouts (Table 9).

### 5.2 Lying time and milk yield

In Experiment 6, the mean milk yield (SD) in the morning was 19.5 kg (4.2) and in the evening 18.8 kg (4.1), and the mean daily yield was 38.3 kg (7.8). Higher-yielding cows spent a longer time feeding and ruminating while standing, and less time lying (Figure 4). Yield had no effect on any other measured behavior over 24 h.
Figure 4  Duration of lying and ruminating while standing in relation to milk yield during Experiment 6. All the cows were in their 8th week of lactation. (ruminating while standing $P = 0.002$; slope 7 min/kg, CI 3 to 11 min; lying $P = 0.022$; slope -10 min/kg, CI -19 to -2 min)

Table 10  The 24-hour time budget of primiparous and multiparous cows kept in tie stalls in Experiment 6 (mean ± SE). Letters indicate a significant difference between parity classes ($P < 0.05$).

<table>
<thead>
<tr>
<th>Minutes per 24-hour</th>
<th>Primiparous N = 14</th>
<th>Multiparous N = 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>589 ±48</td>
<td>685 ±47</td>
</tr>
<tr>
<td>Eating</td>
<td>225 ±13</td>
<td>211 ±12</td>
</tr>
<tr>
<td>Ruminating</td>
<td>372 ±18</td>
<td>435 ±17</td>
</tr>
<tr>
<td>Lying while ruminating</td>
<td>161 ±31</td>
<td>282 ±31</td>
</tr>
<tr>
<td>Standing while ruminating</td>
<td>208 ±27</td>
<td>141 ±27</td>
</tr>
<tr>
<td>Other activities</td>
<td>502 ±30</td>
<td>516 ±30</td>
</tr>
<tr>
<td>Lying with other activities</td>
<td>97 ±21</td>
<td>127 ±20</td>
</tr>
<tr>
<td>Lying inactive without ruminating</td>
<td>206 ±29</td>
<td>192 ±28</td>
</tr>
<tr>
<td>Lying with the neck relaxed</td>
<td>109 ±12</td>
<td>82 ±12</td>
</tr>
</tbody>
</table>

Before and after milking, the milk yield affected cow behavior (Table 11). Cows with a higher milk yield had a shorter latency to lying inactive without ruminating or with the neck relaxed ($P = 0.020$; slope -0.5 min/kg).
Table 11  Percentage allocation of time to different behaviors 4 hours before and after milking in Experiment 6. The effect of milk yield on behavior is indicted by arrows (↑, P < 0.05). Each time period is analyzed separately.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>2 to 6</th>
<th>6 to 10</th>
<th>14 to 18</th>
<th>18 to 22</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lying</td>
<td>38 ↓</td>
<td>60 ↓</td>
<td>22</td>
<td>50 ↓</td>
</tr>
<tr>
<td>Eating</td>
<td>13 ↑</td>
<td>11 ↑</td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>Standing without eating</td>
<td>49 ↑</td>
<td>29</td>
<td>55</td>
<td>33 ↑</td>
</tr>
<tr>
<td>Ruminating</td>
<td>34</td>
<td>24</td>
<td>19</td>
<td>24</td>
</tr>
<tr>
<td>Lying while ruminating</td>
<td>-</td>
<td>18</td>
<td>-</td>
<td>15 ↓</td>
</tr>
<tr>
<td>Standing while ruminating</td>
<td>-</td>
<td>6</td>
<td>-</td>
<td>9 ↑</td>
</tr>
<tr>
<td>Lying inactive without ruminating</td>
<td>11</td>
<td>22</td>
<td>9</td>
<td>19</td>
</tr>
<tr>
<td>Lying with the neck relaxed</td>
<td>6</td>
<td>8</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Other activities</td>
<td>35</td>
<td>35</td>
<td>46</td>
<td>32</td>
</tr>
</tbody>
</table>

↑ = positive association between behavior and yield  
↓ = negative association between behavior and yield

5.3 Stall surface preferences

The preferences between two stall bedding materials were tested with preference tests (Figure 5). In Experiment 1, in the comparison between sand and straw, the straw stalls were occupied more often than sand stalls. For the comparison between rubber mats and sand stalls, the rubber mat stalls were occupied more often than sand stalls (in all groups) in winter, while in summer the rubber mats were preferred over sand stalls in three groups.

In the comparison between rubber mats and straw, the straw stalls were occupied more often than rubber mat stalls in winter in 3 out of 4 groups of cows. In summer, there were no differences in preference between straw and rubber mats.

In the Experiment 2, the cows showed no preference for lying in stalls with fine sand, rough sand or for lying in the concrete alleyway of the cow house.

In Experiment 4, cows having concrete flooring with straw bedding as their previous bedding material preferred to lie in stalls with straw bedding (Figure 5). However, cows that had previously been kept on sand did not show a preference for sand or straw.

In Experiment 5, in the preference test comparing rubber mats and concrete stalls, cows showed no overall preferences between sand and concrete-based stalls or between rubber mat and sand stalls. However, cows were observed to lie down more often on rubber mats than in concrete stalls (Figure 5).
Figure 5  The preferences revealed by preference tests. Not all the materials were tested during all the experiments. There were several differences between the experiments, for example in the amount of bedding. Concrete flooring is referred in the figure as straw since it was never unbedded. In Experiment 2, there was no difference in preference between fine (54%) and rough (46%) sand, and the data are not shown in figure. *(P < 0.05)

5.4 Stall surface and health

Injury and hoof health

The severity of hock lesions decreased over time (*P* < 0.001), and there was an interaction between time and material in hock lesion size (*P* = 0.04, Table 12). At the end of Experiment 3, the severity of hock lesions was lower for cows on sand than for those on straw. There were no differences between cows in straw or sand stalls in terms of changes in the circumference of the knees or the size of hock lesions.

Table 12  Average hoof lesion score (± SE) of cows using straw (N=17) or sand stalls (N=17) at the beginning and end of the experiment. In the division of types of hoof lesions found, each claw half was recorded separately. The average size of hock lesions on the back legs, as well as the average (median, interquartile range) severity scores of hock lesions on cows using straw-bedded or sand stalls at the beginning and end of at least a 21 week epoch in Experiment 3 are presented.

<table>
<thead>
<tr>
<th></th>
<th>Straw</th>
<th>Sand</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beginning</td>
<td>End</td>
</tr>
<tr>
<td>Hoof lesion score</td>
<td>5.0 ± 0.48</td>
<td>5.3 ± 0.39</td>
</tr>
<tr>
<td>Sole hemorrhaging</td>
<td>46</td>
<td>49</td>
</tr>
<tr>
<td>Heel horn erosion</td>
<td>51</td>
<td>51</td>
</tr>
<tr>
<td>White line disease</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Other hoof disease</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Hock lesion size, mm²</td>
<td>710 (130)</td>
<td>518 (113)</td>
</tr>
<tr>
<td>Hock lesion severity</td>
<td>2.0 (1.0 to 3.0)</td>
<td>1.0 (1.0 to 2.0)</td>
</tr>
</tbody>
</table>

*Maximum hoof lesion score is 8, 0 = healthy.
*Hock lesion severity scores on a scale of 0 to 5, where 0 = healthy.
*Superscripts indicate a significant difference (*P* < 0.05)

Cleanliness

In Experiment 3, there were no differences in dirtiness between stalls with different bedding materials. However, the cows using straw stalls were dirtier than those using sand stalls [6.04 (5.39 to 6.28) vs. 4.19 (3.62 to 5.16), *P* < 0.001]. The difference was most prominent for the midlegs, upper legs, and belly.
6 Discussion

The dairy cows spent an average of 4 to 12 h per day lying down during the experiments in free stall-housing (I-III) and 11 h per day in tie-stall housing (IV). The lying times in this study were within the range normally reported (Jensen et al. 2005), except for the experiments of a few weeks duration on sand bedding without any organic materials. In a study of 43 free-stall farms, a lying time of 11 hours per day was observed by Ito et al. (2009). The lying, feeding and ruminating times in tie stalls (paper IV) were also comparable with earlier reports (Dado & Allen 1994, Alzahal et al. 2006).

Lying behavior was elicited by the organic straw bedding (I, II, III). However, there was large individual variation in the preference for the bedding material, possibly caused by intrinsic factors such as health status, milk yield, or age. Ito et al. (2009) observed that variation in lying behavior was greater between individual cows than between farms.

When given a free choice between different stall bedding materials, cows could indicate the relative comfort level through their preference.

<table>
<thead>
<tr>
<th>Which stall do cows prefer: straw covered concrete, rubber mats or sand?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cows preferred straw compared to sand</td>
</tr>
</tbody>
</table>

Cows avoided sand, preferring straw and soft rubber mats both in the winter and summer (I). The preference for materials other than sand is in accordance with the findings of Shim et al. (1998) and Tucker et al. (2003).

Cows preferred straw-covered concrete to rubber mats in the winter, but in the summer there was no difference (I). Straw provides insulation thus reducing thermal discomfort (Nilsson, 1988, Tuyttens 2005), and the preference for straw may therefore be due to its thermal properties. This suggestion is supported by the fact that the preference for straw was less obvious in the summer (I). In agreement with this, Gebremedhin et al. (1985) found that cows used concrete stalls with a thin layer of bedding more in the summer than the winter.

Our results (II) are consistent with those of Natzke et al. (1982), Herlin (1997), and Chaplin et al. (2000b), who found that cows lie down for longer on softer materials. Tucker et al. (2003) noted that cows preferred organic sawdust bedding to sand.

The most striking finding was that sand bedding was clearly the least preferred material compared to the other alternatives (I). This led to the need to more deeply explore the effect of sand bedding quality.

<table>
<thead>
<tr>
<th>Is the sand particle size of importance for stall preference?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possibly not, because there was no difference in preference between two particle sizes</td>
</tr>
</tbody>
</table>

It has been reported that the ability of sand bedding to adjust to the form of lying animals depends on the size of the sand particles (Ekelund et al. 1998). However, no preference was found for fine or rough sand, which indicates that the particle size of the sand was not
the reason for the cows rejecting rough sand when straw or rubber mats were available. There have been no previous studies evaluating the preference of cows for different sand qualities.

Sand as a stall bedding material is recommended because of its hygienic properties (Britten 1994, Niles 1994, Godden et al. 2008), the advantages in reducing hock injuries (Weary & Taszkun 2000), and the reduced costs of earth-based sand stalls compared to concrete ones (Ekelund et al. 1998). It is also claimed to be more comfortable for cows (Ekelund et al. 1998, Cook 2003). In addition, the use of sand bedding has been found to improve hoof health (Espejo et al. 2006).

Despite the reported advantages of sand as a bedding material (Cook et al. 2008), a longer lying time or lying preference for sand has not been reported in the literature on sand bedding compared to organic bedding materials. Our findings (I, II) in preference tests indicated that cows strongly avoided using sand when other soft materials were available, suggesting that cows did not find sand a comfortable bedding material. This was confirmed by the observations on lying time, as the time spent lying down was shorter on sand compared to other materials (I, II). Consistently, with this, Bewley et al. (2010) observed a lying time of only 10.5 hours on sand-bedded stalls. Even when a small amount of bedding was used, the cows showed no preference for sand stalls over stalls with concrete floors; nor were resting times increased (III).

There are a number of possible reasons why our cows avoided sand-bedded stalls. The observation of the same preference in both winter and summer (I), despite the large temperature difference in the barn, suggests that the thermal properties of sand may not be the key factor.

Sand moves and can be unstable (Ekelund et al. 1998), which may be uncomfortable for cows that are used to a firm concrete stall base. Cows might have had difficulties in coming out of the sand stalls while stepping over the rear curb (29 cm). Ekelund et al. (1998) found no difference in lying down behavior in cows lying on a sand–sawdust mixture or on soft rubber mats with sawdust bedding. This suggests that sand does not make it more difficult to get up or lay down. Moreover, Cook and Nordlund (2009) suggest that sand bedding could even facilitate the rising and lying down movement of lame cows.

The avoidance of sand may be due to its unfamiliarity to the cows, as naïve cows avoided sand in our 3-week experiments. However, the long-term effects of sand bedding need to be investigated before considering it an unsuitable bedding material.

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**Does the familiarity with the stall surface affect the lying time?**

No, the results of short- and long-term experiments were the same.

---

In agreement with the results of a short (3 day) experiment (I), a longer total duration of lying down in straw-bedded stalls compared to sand stalls was apparent during the 21-week observation period (II).

During the adaptation week at the beginning of the experiment, the cows with straw-bedded stalls lay down for longer than those with sand-bedded stalls. This was especially
marked during the first day, but the cows rapidly adapted and there was much less of a difference during the subsequent days (II).

Whether the reduction in lying time on sand (II) is sufficient to affect the welfare of the cows is not clear: the durations of lying in both sand and straw-bedded stalls were within the range of lying times usually reported (Tucker et al. 2003, Cook et al. 2004). However, Jensen et al. (2005) concluded that heifers need 12 to 13 h of resting time daily. In our experiment the cows spent 11 h per day lying down on sand (II).

Our findings were consistent during short- and long-term experiments; however, we were also interested in assessing the effect of earlier exposure on the preference test results.

<table>
<thead>
<tr>
<th>Does the familiarity with the stall surface affect the preference?</th>
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<td>Yes, cows prefer familiar materials</td>
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Cows tended to prefer straw-bedded stalls if they had previously been kept in such stalls. Prior familiarity with sand increased the preference for sand, but did not lead to greater preference for sand over straw.

Previous experience has been shown to affect the choices of animals in preference tests (Duncan 1992, Grandin et al. 1994), and familiarity of the flooring material may be important for cows. Sonck et al. (1999) found that cow preferences for certain mattresses became more distinct during their experiment.

Our results confirmed that previous experience plays an important role in the preference for bedding materials (I, II). Tucker et al. (2003) reported that exposure to sand during two previous lactations made sand roughly as desirable as sawdust for lying, but exposure of just a few days did not affect their preference so thoroughly. In our preference tests, cows avoided lying on sand stalls without bedding compared to other materials (I, II). However, extended earlier experience of sand increased the acceptance of sand although bare sand never appeared to be a preferred lying surface material.

To attain greater acceptance of sand-bedded stalls, cows need to have time to get used to the material. However, more studies are needed to determine whether raising young animals on sand would affect their preferences, because the animals we used did not have access to sand as calves.

Before our experiments began, the cows were lying on hard rubber mats on concrete with a mixture of peat and cut straw on them, and between the different comparisons they were housed on concrete or plywood placed on sand and bedded with peat. Between Experiments I to III, the cows had been housed in an insulated or an uninsulated barn with concrete-based stalls having hard rubber mats and softer mats and mattresses bedded with a mixture of peat and cut straw. During the summer the cows were kept on pasture. These experiences could have affected the results. Ideally, all the cows would have been raised and kept on the same control surface material, and one that was not included in the experiments.

Cows in groups that went through the no-choice phase and used sand stalls for three days tended to lie on sand more than cows that were not persuaded to try it (I). Furthermore, cows used sand stalls more in the sand quality experiment after having
experienced sand stalls. This indicates that the degree of familiarity had an effect on the acceptance of sand. However, although some of the cows seemed to learn to use sand stalls, there were some cows who never used them or lay down both in alleyways and in the stalls (I).

Overall, sand was the least preferred bedding material; however, to obtain a wider perspective on the welfare effects of different stall bedding materials, we also investigated some health issues associated with long-term exposure to the same materials. We were also interested in determining whether the cows could make good choices for their wellbeing.

<table>
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<tr>
<th>Do cows choose a material that benefits their health?</th>
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<tr>
<td>No, on the contrary, sand benefits leg health</td>
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Despite the behavioral preferences, cleanliness and hoof health were better for cows kept in sand-bedded stalls than those kept in straw-bedded stalls during the 21-week experiment (II). In addition, hock lesions healed more rapidly on the cows using sand stalls. The tendency of sand bedding to conform to the body shape of the cows might be the reason for the improved healing, because a lying cow can position her leg to avoid pressure on a lesion. Vokey et al. (2001) suggested that less contact with bed surface increased healing. Our results are in agreement with Weary and Taszkun (2000), who found a lower incidence of hock lesions on sand than on geotextile mattresses or sawdust, and with Vokey et al. (2001), who observed the best recovery on sand compared with mattress and concrete flooring. Fulwider et al. (2007) also recorded fewer cows with lesions on sand compared with rubber-filled mattresses. Claw disorders of cows using sand recovered more compared with straw (II). Our results are consistent with previous findings (Cook 2003, Cook et al. 2004, Espejo et al. 2006) of a lower prevalence of lame cows in sand stall herds compared with those housed with mat stalls.

There is a trade-off between improved hoof health and reduced cow comfort with the use of sand bedding. Cows preferred straw to sand bedding and lay down longer on straw covered-concrete, whereas cleanliness and hoof health were better on sand (II). The leg health issue is only relevant with lame animals, but as the hoof or skin lesions can cause severe pain to the affected individuals, the issue is still important for welfare. It is surprising that the use of the preferred stall material did not translate into health benefits. After all, a material that enhances wound healing would be assumed to be more comfortable. Nonetheless, leg health is only a limited measure of a cow’s overall health, and we did not subject the cows to full veterinary examination. In addition, the number of cows that were included in leg health observations was quite small to obtain definitive results on the existence of rare hoof lesions. In further studies it would be interesting to investigate whether sand combined with straw or with some other soft material could convey health benefits and at the same time maintain the lying time.

The association between claw health and lying time is complex. Cook (2003) suggested that the benefits of sand as a bedding material may come from its ability to increase stall use and recumbency time. However, according to our results, the improved claw health in sand stalls was not due to an increased time spent lying down in the stalls.
In contrast, a reduced hoof health of cows using straw bedding might have been responsible for the increased lying time of cows kept on straw-bedded stalls, because lame cows have been found to spend more time lying down (Singh et al. 1993b, Galindo & Broom 2002, Juarez et al. 2003). However, the connection between claw health, injuries, and daily lying time is complex, as some papers have reported that claw lesions were associated with a reduced lying time (Singh et al. 1993a, Chaplin et al. 2000a, Cook et al. 2004). Moreover, Singh et al. (1994) suggested that a longer lying period might be important for the prevention of lameness. Nevertheless, in our experiment (II), sand bedding improved hoof health and reduced the time spent lying.

In addition to leg health, stall material is considered to affect the cleanliness of cows, and thereby the susceptibility to mastitis.

<table>
<thead>
<tr>
<th>Which stall material enhances cleanliness?</th>
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<tbody>
<tr>
<td>Sand</td>
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</table>

There was no difference between the cleanliness of stalls; however, cows in straw stalls were dirtier compared with those in sand stalls (II). The results of the preference tests need to be interpreted considering the different amounts of straw bedding that were used to cover the surface material.

<table>
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<th>Is the amount of straw of importance for the preference?</th>
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<td>Yes, cows prefer more straw and soft bedding material</td>
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In general, the cows preferred the stalls with the most bedding, as adding straw on sand increased its use and reducing the amount of straw on concrete decreased its use (I, III). The amount of bedding seems to affect the lying preferences of cows. The insulating and softening properties of straw, among other factors could make it preferred by cows.

Cows reclined for longer in stalls with soft rubber mats compared to sand- and concrete-based stalls when provided with only a small amount of straw in the free stalls (III). This supports the results of O’Connell and Meaney (1997), who showed that cows preferred rubber mats to concrete with the same kind of sawdust bedding. Herlin (1997) reported that cows preferred soft rubber mats to conventional, hard rubber mats or concrete when 2-3 kg of bedding was added per stall. Furthermore, the resting time was greatest in the stalls with rubber mats during the no-choice period (III). Our results support those from the study of Rushen et al. (2007), in which cows tended to rest for longer on rubber mats compared to concrete-based tie-stalls with 0.5 kg straw bedding. In addition, concrete covered with only a small amount of bedding causes knee swellings (Rushen et al. 2007), which indicated an uncomfortable resting surface. Moreover, Haley et al. (2000) found soft rubber mattresses to increase the duration of lying compared to a concrete surface when only a small amount of straw was used.

The use of soft rubber mats may provide a means for bedding management with only a small amount of straw while maintaining cow comfort in unheated buildings. The air layer underneath of rubber mats could improve their thermal properties during the winter.
The cows preferred concrete with straw to soft rubber mats in winter (I). Having a large amount of straw on concrete may have made the straw stalls softer than mat stalls, which may have affected the cows’ preferences. Gebremedhin et al. (1985) found that increasing the depth of bedding on concrete increased stall use. It has also been shown that cows prefer concrete with 15 cm of sawdust to different kind of mats without bedding (Nilsson 1988). Tucker et al. (2009) concluded that cows preferred organic bedding, whether straw or sawdust.

The combined findings of papers I and III, in which the rubber mat stalls were very similar, show that cows preferred the stalls with the softest flooring, which could either be achieved by using deep straw bedding or by the use of soft rubber mats. Adding straw onto sand increased its use relative to mats, and reducing the amount of straw on concrete decreased its use relative to mats. We found that the amount of bedding affects the lying preferences on concrete and sand (I, III). This is in accordance with and extends the findings of Tucker and Weary (2004) and Tucker et al. (2009) who observed that the amount of bedding has an effect on lying on mattresses. The insulating and softening properties of straw can make it preferred by cows (I, III).

Installing soft rubber mats on concrete stalls can ameliorate the adverse effects of reduced bedding on lying behavior. Lying times on mats indicates that they were comfortable for the cows compared to concrete and sand bases (III). It appears that when a small amount of straw is available cows lie down more on soft rubber mats. In addition, newly laid or fresh straw has been observed to stimulate lying down in the short term (II).

The longer resting time was due to a higher frequency of lying bouts on straw and rubber mats than on sand (I). A similar effect of softer stall flooring in increasing the frequency with which cows lie down and stand up was previously noted in tie stalls (Haley et al. 2001, Rushen et al. 2007). In contrast, Cook (2003) suggested that sand may help cows to rise and lie down by improving the surface traction in the stalls.

Due to complex connections between lying time and hoof health, it is difficult to interpret lying time alone as a measure of the effect of stall design on animal welfare, unless we understand the reasons for the differences in time spent lying down. The large variation between cows in their preferences and resting time led us to look closer at the potential factors affecting the behavior. The effects of intrinsic factors such as milk yield on cow lying behavior need to be illuminated.

<table>
<thead>
<tr>
<th>Does milk yield affect the lying time?</th>
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<tbody>
<tr>
<td>Yes, cows with a higher milk yield lie down less</td>
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Cows with a higher milk yield spent less time lying (IV) suggesting that other factors besides time constraints due to the increased duration of feeding affect the lying behavior. High-producing cows at their peak lactation spent less time lying per day than lower producing cows, in agreement with Hasegawa et al. (1993). Milk yield changes according to the stage of lactation, and our results (IV) are indirectly supported by previous results showing that cows in the early stage of lactation lie down for a shorter total time than

In the morning, cows with a higher milk production spent less time lying before milking (IV). Discomfort of the udder is one possible explanation suggested by Österman and Redbo (2001) and Overton et al. (2002). A standing posture while anticipating milking ultimately represents maternal behavior, as a cow permits calf suckling by standing still (Kohari et al. 2004). Furthermore, Tucker et al. (2007) found an increase in udder firmness scores when the milking frequency was changed from twice a day to once a day.

However, during other times the results were difficult to interpret (IV). There was no effect of milk yield on lying time before the evening milking, while after both milkings, cows with a higher yield spent a shorter time lying. Thus, the results suggest that other factors besides the anticipation of milking or the long time needed to consume feed also affect the duration of lying. After the evening milking the higher-yielding cows were observed to be lying less and standing more while ruminating. Moreover, cows with a higher milk yield generally spent more time ruminating while standing (IV).

To assess whether a reduced lying time increases motivation to sleep, we inspected the latency to lie with the neck relaxed.

| Is latency to relax the neck after lying down affected by milk yield? |
| Yes, high yielding cows more rapidly lie with the neck relaxed |

High producing cows started lying with the neck muscles relaxed sooner within lying bouts than lower producing cows (IV). According to Ruckebusch (1975) and Hänninen et al. (2008), lying with the neck muscles relaxed as well as being inactive and without ruminating are signs of sleep. Cows generally started their lying bouts with active lying behaviors, and lying inactive without ruminating took place earlier than ruminating in the course of an average lying bout (IV). The latency to sleep (lying with the neck relaxed or inactive without ruminating) within lying bouts decreased with increasing yield. This suggests that high yielders might have been more motivated to sleep than lower yielding cows and were intensifying the lying bouts. Ruckebusch (1974b) showed that after sleep deprivation, cows were more motivated to sleep and slept more. In addition, sleep latency is commonly used to evaluate sleepiness in other species (McKenna et al. 2008) and basic functions of sleep are believed to be shared at least between mammalian species (Tobler 1995). Several authors have reported that cows, after a few hours of forced standing, spend a longer time lying than cows with free access to lie down (Metz 1985, Cooper et al. 2007, Cooper et al. 2008).
7 Conclusions

In these studies, cows were found to respond to different bedding materials by changes in their lying behavior, usually preferring softer stall surface materials. In addition, cows were observed to change their behavior in association with the increasing milk yield. A high milk yield affected resting by reducing the lying time, and by shortening the time taken to fall asleep. Therefore, the management of highly productive cows should consider the high motivation to eat and also the high motivation to sleep and rest. Ideally, routines and facilities should avoid any unnecessary standing, for example when waiting for access to the milking station or to feeding and resting areas. Whether the behavior of high-yielding cow resembles that of resting motivated cows needs to be investigated. The effects of genetic selection on behavior and motivation should be elucidated in further studies. Cows in loose housing systems are affected by more pronounced time constraints because they need to walk between lying and feeding areas and wait for access to milking compared to cows kept in tie stalls.

It is possible that individual cows have different needs for their lying environment due to intrinsic factors affecting their behavior. Adding fresh bedding material to stalls increases the comfort of the stall flooring materials. Despite preferences for straw, leg health and cleanliness seems to be improved by the use of sand stalls. Therefore, it would be interesting to investigate whether a mixture of straw and sand could be perceived as comfortable as well as being beneficial for leg health.

Table 13 The lying time of a cow is affected by the innate state of the animal and its environment. These experiments have illuminated the effect of some of the factors. The research questions and the most important findings of this study are presented.

<table>
<thead>
<tr>
<th>Question</th>
<th>Finding</th>
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<tbody>
<tr>
<td>Which stall do cows prefer: straw-covered concrete, rubber mats or sand?</td>
<td>Cows preferred straw compared to sand</td>
</tr>
<tr>
<td>Is the sand particle size of importance for stall preference?</td>
<td>Possibly not, because there was no difference between the two particle sizes</td>
</tr>
<tr>
<td>Does familiarity with the stall surface affect the lying time?</td>
<td>No, the results of short- and long-term experiments were the same</td>
</tr>
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I wish to express my gratitude to my supervisors, Lene Munksgaard and Professor Anna Valros, for your drive for science, enthusiasm about animal welfare and persistence in general. I had the privilege to get to know Lene and her family when I was invited as a guest to their beautiful country home during my visits to Denmark. Anna Valros has been my dear friend even before becoming my supervisor.

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I wish to thank my family for enabling me to devote my time to studies; thank you mother for looking after Marialina, and Marialina for taking such a good care of Sessu the dog. I also owe my sincere gratitude to all my old friends that supported me and had almost unrealistic admiration of my studies during the years.

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References


