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Doctoral Dissertation

Jutta Johanna Kauppi

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Abstract

Animal welfare includes both physiological and mental health and is affected by several external and internal factors. In dairy cows, human care and the ability of the cows to cope with daily challenges are the most significant factors. The research presented in this dissertation focused on cow behaviour and aspects impacting on behavioural changes during mastitis and milking. To deepen knowledge of the relationship between the behaviour and health of cows and associated detection methods, we experimentally manipulated the health status of dairy cows through mastitis induction. Moreover, we tested and validated a thermal infrared camera for recording the udder skin temperature, which could be helpful in the case of early mastitis detection. Furthermore, we examined the relationship between cow behaviour and milking in herringbone and automatic milking systems. We established that cow behaviour changed during mastitis. The most apparent changes were in lying, eating and stepping behaviour. It was shown that inflammation affected the cow health status, which changed the behavioural priorities. In contrast to our expectations, visual signs in the udder and changes in milk composition occurred only 2 hours post-challenge, while clinical and behavioural changes were first recorded 4 hours post-challenge. However, changes in lying and restlessness behaviours were promising indicators for detecting signs in cows exposed to mastitis. The transient increase in body temperature of cows with experimentally-induced clinical mastitis was successfully detected by udder skin temperature detection with the help of a thermal camera. The udder skin temperature rose simultaneously with the rectal temperature. However, local inflammatory changes in the udder, appearing earlier than the rectal temperature increase, were not detected with udder skin temperature measurement by using the thermal infrared camera. Regarding cow behaviour as an indicator of the success and quality of the milking process, we found that half of the deviations occurring during automatic milking originated from cow behaviour, such as cows kicking, lifting their legs and moving during milking, and had their origins in machine failures. To conclude, cow behaviour can be used as an indicator for detection of mastitis and (un)successful milking. However, effectively functioning human-animal-technology interactions should be studied more in the future in order to enhance husbandry practices that can improve animal welfare.

Lehmän käyttäytymisen yhteys eläimen terveydentilaan, hyvinvointiin ja lypsyyn

Eläimen hyvinvointi muodostuu sekä fyysisestä että psyykkisestä tilasta ja siihen vaikuttavat lukuisat eläimen sisäiset mutta myös ulkoiset tekijät. Lypsylehmän hyvinvointiin vaikuttavista tekijöistä tärkeimpiä ovat karjanhoitajan hoitotoimenpiteet sekä lehmän kyky sopeutua päivittäisiin haasteisiin. Tässä väitöskirjatyössä keskitytään lehmän käyttäytymiseen sekä käyttäytymisen muutoksiin, jotka liittyvät lypsyyn tai lehmän terveydentilan heikkenemiseen.

Keskityin selvittämään lehmän käyttäytymisen, hyvinvoinnin sekä terveydentilan tunnistamiseen liittyvien menetelmien välistä vuorovaikutusta. Vaikutimme keinotekoisesti lehmän terveydentilaan ja saimme näin uutta tietoa siitä, kuinka utaretulehdus muuttaa lehmän käyttäytymistä yksilön terveydentilan heiketessä. Tämän lisäksi testasimme ja validoimme samanaikaisesti uuden teknologian (infrapunakameran) käyttöä utareen pintalämpötilan tunnistamisessa. Tavoitteenamme oli tehokkaampi alkavan utaretulehduksen tunnistaminen karjoissa. Tutkimme myös lehmän käyttäytymisen ja lypsyn onnistumisen välisiä yhteyksiä sekä kalanruotoettä automaattilypsyssä.

Lehmä muuttaa käyttäytymistään terveydentilan heiketessä, sairastuessaan utaretulehdukseen. Selkeimmät muutokset havaittiin makuu- ja syömiskäyttäytymisessä - sekä levottomuus (jalkojen nostelu) käyttäytymisessä. Lehmän käyttäytymisen prioriteetit muuttuvat sen sairastuesa utaretulehdukseen. Toisin kuin odotimme, maidon koostumuksen silmin nähtävät muutokset voitiin tunnistaa jo kaksi tuntia utaretulehdukseen sairastumisen jälkeen, kun taas kliiniset- sekä käyttäytymisen muutokset voitiin tunnistaa vasta neljä tuntia lehmän sairastumisen jälkeen.

Lehmän makuukäyttäytyminen ja levottomuus (jalkojen nostelu)käyttäytymisen muutokset osoittautuivat tutkimuksessamme lupaaviksi indikaattoreiksi tunnistettaessa utaretulehdukseen sairastuvia lehmiä. Lehmän ruumiinlämpötilan lyhytaikainen nousu kokeellisesti aiheutetun utaretulehduksen aikana tunnistettiin infrapunakameralla utareen pintalämpötilaa mittaamalla. Utareen lämpötila kohosi samanaikaisesti lehmän rektaalilämpötilan kanssa. Kuitenkin paikalliset tulehdusmuutokset utareessa ilmenivät ennen rektaalilämpötilan nousua, eikä niitä kyetty tunnistamaan infrapunakameran avulla.

Puolet automaattisen lypsyn aikaisista häiriötilanteista oli lehmän käyttäytymisen aiheuttamia (potkut, jalkojen nostelu, liikehtiminen lypsyn aikana) ja lehmän käyttäytymisellä oli selvä vaikutus lypsytapahtuman onnistumiseen sekä lypsyn kunnolliseen läpivientiin.

Lehmän käyttäytymistä sekä sen muutosta voidaankin käyttää indikaattorina niin utaretulehduksen tunnistamisessa kuin myös lypsyn onnistumisessa.

Jatkotutkimuksia tarvitaan, jotta ihmiseläin-teknologia-vuorovaikutus saadaan mahdollisimman hyvin toimivaksi niin, että käytännön tilalla lehmän hyvinvoinnin tarkkailu sekä terveydentilan heikkenemistä ehkäisevät karjanhoidon ratkaisut saadaan tehokkaiksi. Näin voidaan ennaltaehkäistä lehmän terveydentilan heikkenemistä ja edistää lehmän hyvinvointia.

Lehm ja koiv

Mää tahro olla lehm koivu al.
Mää en tahro olla luav.
Mää en tahro oppi uut taitto-ohjelma.
Mää en tahro selvittä äit-tytär suhret.
Mää en tahro viärä sitä kirjet posti.
Mää en tahro soitta Kelan tätil.
Mää en tahro muista yhtäkän pin-koori.
Antakka mu olla lehm koivu al.
Viäkkä mu väsyne nahk kamarim permanol, kakluni ette.

The Cow and the Birch Tree

I want to be a cow under the birch tree
I don't want to be creative
I don't want to learn a new software program
I don't want to sort out the relationships of the mothers and daughters
I don't want to take that letter to the post office
I don't want to phone that woman at the benefits office
I don't want to memorise any more pin numbers
Take my tired hide and put it in front of the hearth

Heli Laaksonen (Pulu uis 2000)
(englanniksi kääntäneet Mark Phillips ja Christa Prusskij)

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I thank my lovely daughter Alisa for her patience and reminding me that this is a project to be finalized in order to be able concentrate on more important issues in life: playing and having fun.

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List of original publications

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II. Hovinen M., **Siivonen** J., Taponen S., Hänninen L., Pastell M., Aisla A-M., and Pyörälä S. Detection of clinical mastitis with the help of a thermal camera, Journal of Dairy Science, 2008, 91, 12: 4592-4598. doi:10.3168/jds. 2008-1218.

III. **Kaihilahti** J., Suokannas A. and Raussi S. Observation of cow behaviour in an automatic milking system using web-based video recording technology. Biosystems Engineering 2007, 96, 1: 91-97. doi:10.1016/j.biosystemseng. 2006.10.001.

IV. **Siivonen** J., Pastell M., Aisla A-M., Jauhiainen L., Ahokas J., Vainio O. and Raussi S. Effect of milking and management on cow behaviour. Submitted.

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Contents

1 Introduction

The current trend in Europe is a constantly increasing farm and average herd size. At the same time, the use of new technology in the barn and automation of certain activities is growing accordingly. For example, increasing numbers of dairy farms are switching from conventional twice per day milking systems to milking robots (automatic milking system, AMS). Furthermore, additional tools and devices can be added in order to automatically detect, for example, lameness (e.g. Pastell et al., 2008) or mastitis through the conductivity of the milk. Some of these tools do actually provide information to stockpersons, allowing them to improve cow welfare. However, the information provided by these tools is not always easy for stockpersons to interpret and is based on physiological (e.g. milk conductivity) or physical changes (e.g. weight pressure of feet on a mat). Measures might be taken too late in certain cases, and more modern observation technology is needed to provide further online information and to observe changes in the behaviour of individual cows and welfare in the barn. The importance of monitoring cow welfare will increase as direct human–cattle contact during milking disappears with the change from conventional milking to automatic milking systems (AMS). As daily human–cattle interaction decreases, other ways of observing changes in the welfare of cows will become more prominent.

Mastitis is one of the most common diseases of high-producing dairy cows, and acute mastitis is the most important welfare and economic problem in dairy herds (Halasa et al. 2007). A recent study by Heikkilä et al. (2012) demonstrated that it is most profitable to treat mastitic cows and keep them in the herd as long as healthy ones. This supports the fact that more intensive observations on both behavioural and clinical changes within the herd are needed to detect and react to ongoing changes in cow welfare.

Cows that develop mastitis display both behavioural and clinical changes (Kemp et al. 2008, Rousing et al. 2004). Acute mastitis can cause motivational conflict in the behavioural priorities of a cow, and thus change the classical patterns of sickness behaviour. However, there is still a lack of knowledge concerning which precise behaviours change first at the beginning of acute mastitis, and whether behavioural changes might serve as a tool for its early detection.

In general, numerous factors affect the behaviour and welfare of dairy cows, with human care and the production environment being the most significant. This thesis focuses on cow behaviour and the capacity of cows to adapt to challenges faced in daily life, such as mastitis and milking.

Previously, several studies have reported the effects of different dairy management regimes on cow behaviour. Total lying time and the number of lying bouts, or standing in the lying area, have been used as indicators of cow welfare (Cook et al. 2005, Fregonesi and Leaver 2001, Haley et al. 2000). In addition, changes in lying down and rising movements as well as cow comfort in resting have been used to detect changes in cow welfare (Lidfors 1989, Plesch et al. 2010).

Motivational priorities play a key role in affecting behavioural responses. For example, fear competes with sickness, and fear-motivated behaviours take precedence over sickness-motivated behaviours (Aubert 1999). Sickness behaviour is considered to be more an expression of the motivational state than a consequence of weakness (Miller 1964). Sick animals exhibit distinct behavioural patterns; a sick individual shows decreased activity, exploration, body care and sexual behaviour, as well as having a poor appetite (International Dairy Federation 1999). In severe cases, rumen contractions of sick cows slow down (Radostits et al. 2007). If sickness behaviour is prevented, recovery from disease is poorer (Johnson 2002). The research presented in this thesis investigated the effect of mastitis on cow behaviour, which altered the normal behavioural patterns, thus resulting in changes in behavioural priorities (I).

The results have improved our understanding of how a cow modifies its behaviour when infected with mastitis. It was found that the detection of changes in behavioural patterns can serve as an additional tool when detecting clinical mastitis. A study by Fogsgaard et al. (2012) confirmed that cows do react to pain by altering their behaviour, including changes in milking. As milking in automatic systems is carried out by machines, the stockperson does not have information on changes in a cow's behaviour during the milking process. I suggest that there exists an intensive interaction between the development of mastitis and changes in cow behaviour. Moreover, success in milking is essential to maintaining the health and welfare of cows, but also has an impact on cow behaviour.

New technologies will continuously be introduced in barns in order to secure online information on cow welfare parameters on a long-term basis. However, there is still a lack of information on the precise behavioural changes that best describe the changing health status in cows and that can be used to detect illnesses as early as possible.

The interactions between humans, animals and technologies will become increasingly important, as individual cow welfare must be ensured, despite the reduced time available to stockpersons to devote to individual animals.

In the following literature review, I discuss some of the factors that affect cow behaviour and welfare. This dissertation focuses on cow behaviour and aspects impacting on behavioural changes during mastitis and milking. To deepen knowledge of the relationship between behaviour and health, and the associated detection methods, we experimentally manipulated the health status of dairy cows by inducing mastitis and observed the behavioural evolution of cows during mastitis development (I). Moreover, we tested a thermal infrared camera regarding its potential in automatic mastitis detection (II). In addition, we observed and compared cows during milking in herringbone and automatic milking systems in order to assess whether cows can easily transfer from one system to another as expressed through their behaviour (III–IV).

II Literature review

Welfare definitions

Animal welfare comprises physiological and mental health and is affected by several external and internal factors (Dawkins 2004, Webster et al. 2004). The welfare of animals is typically addressed by posing three questions: is the animal functioning well, is the animal feeling well and is the animal able to live according to its natural behaviour (Fraser et al. 1997). On a Europe-wide scale, scientists have developed new models based on a multi-criteria-based approach founded on the four main principles of animal welfare: good feeding, good housing, good health and appropriate behaviour (Botreau et al. 2009). This "Welfare Quality (WQ) concept" concentrates on assessing welfare using animal-based criteria, interpreting how the animal performs in its environment.

Cow welfare and coping

To maintain an optimal level of welfare, a cow always attempts to cope with its environment by adjusting its behaviour according to the prevailing circumstances (Broom 1996). When coping fails, signs of poor welfare can occur (Broom 1996). The coping abilities of cows have been assessed using various methods describing the changes in normal, expected behaviour.

Norring et al. (2012) and Drissler et al. (2005) reported the effect of the amount and type of bedding on lying behaviour in cows. Furthermore, the time budget and behavioural preferences of dairy cows were investigated by Munksgaard et al. (2005). Adequate rest (Haley et al. 2000, Munksgaard et al. 2005, Norring et al. 2012) and sleep (Ruckebush 1974) have proven to be essential for cow welfare, in addition to frequent feeding and an adequate water supply. Identified welfare criteria, such as "comfort around resting", have been used by Plesch et al. (2010). Moreover, a decreased total lying time and number of lying bouts or prolonged standing in the lying area have been used as indicators of poor cow welfare (Cook et al. 2005, Fregonesi and Leaver, 2001, Haley et al., 2000). In addition, abnormalities in lying down and rising movements have been used to indicate changes in cow welfare (Lidfors 1989).

Cow behaviour

Cows can alter their behaviour based on their ability to cope with changes in their environment. For example, Albright et al. (1993) found that cows practice social facilitation; they eat more when fed in groups compared to cows fed as individuals. Furthermore, they adapt their feeding speed according to the feeding system (Wierenga and Hopster 1991). Calves and cows (Camiloti et al. 2012, Norring et al. 2012, Tucker et al. 2009) prefer dry, soft and organic lying surfaces that they are familiar with, such as straw compared to sand material. Moreover, cows change their weight distribution by decreasing their rear leg movements while avoiding abrasions of the rear legs and the swollen udder (Chapinal et al. 2013).

The lying time of cows in their time budget is a reliable and relatively consistent welfare indicator that can be followed. A study by Ito et al. (2010) demonstrated that cows spend on average approximately 11 h per day lying down. Lying time is the most prioritised cow behaviour when compared with other behaviours (Munksgaard et al. 2005). Cows do have a strong motivation to lie down (Jensen et al. 2005), but they avoid doing so if the lying surface is uncomfortable (Haley et al. 2001). Several studies have reported changes in lying time to be a relevant indicator of cow welfare (Fregonesi and Leaver 2001, Medrano-Galarza et al. 2012). Fregonesi et al. (2007) found that overstocking reduces the lying time in cows and increases the competition in stalls through cows displacing each other.

Cows and mastitis

Mastitis has a detrimental effect on cow health and welfare because it causes systemic physiological changes, such as an increased body temperature, as well as impacting on milk quality by elevating the somatic cell count (Bannerman et al. 2005). Moreover, it affects cow behaviour in various ways: increasing the hock-to-hock distance (Kemp et al. 2008), increasing restlessness behaviour (Rousing et al. 2004) and decreasing lying behaviour (Haley et al. 2000). Medrano-Galarza and colleagues (2012) observed that mastitis cows display significant differences in behaviour during mastitis days, including a higher frequency of kicks, lifts and steps per minute in milking during the most severe phase of mastitis.

Reactivity and restless behaviour during milking are connected with the discomfort caused by mastitis (Medrano-Galarza et al. 2012). In addition, weight shifting decreased in the rear legs when symptoms of inflammation were at their worst (Chapinal et al. 2013). The weight distribution between the legs and hock-to-hock distance appear to change during the onset of mastitis (Kemp et al., 2008), representing promising indicators of changes in the cow health status (Kemp et al. 2008).

When exposed to mastitis, cows are affected through three levels: 1. the host (i.e. breed, parity and production level), 2. the cause (i.e. pathogen, trauma and chemical) and 3. the environment (i.e. season and management) (Polat et al. 2010). The risk of contracting mastitis is highest during the first month post-partum due to changes in udder adaptation and defence mechanisms and the negative energy balance (Cai et al. 1994).

The effect of automatic milking systems (AMS) on cow health and welfare and their use in detecting changes in the milking process and management has recently attracted wide research interest (Hovinen et al. 2009, Hovinen and Pyörälä 2011, Jacobs and Siegford 2012). Ketelaar-de Lauwere and colleagues (1999) reported the impact of automatic milking on the milking, feeding and resting behaviour of cows. In automatic milking systems, the inspection of foremilk is fully automated and is carried out by a milking robot. Abnormal milk is detected by collecting and modelling the milking data from automatic milking systems (Kamphuis et al. 2008 a and b, 2010, De Mol and Ouweltjes 2001). However, no perfect solution or technological combination for mastitis detection has been established to date. Since mastitis causes marked losses in farm income due to premature culling (Heikkilä et al. 2012), further preventive actions need to be taken to diminish losses at the farm level and to increase longevity in dairy herds. Therefore, effective mastitis detection systems are needed, and it is essential that both clinical and behavioural changes in cows are effectively detected.

Sickness behaviour

Sickness, according to the Encyclopaedia Britannica (2014), is considered as: “A harmful deviation from the normal structural or functional state of an organism. A diseased organism commonly exhibits signs or symptoms indicative of its abnormal state. Thus, the normal condition of an organism must be understood in order to recognize the hallmarks of disease. Nevertheless, a sharp demarcation between disease and health is not always apparent."

Changes in the behaviour of sick individuals are used by veterinarians and stockpersons in the diagnosis of disease (Broom 2006). Sick animals exhibit distinct behavioural patterns, including decreased activity, exploration, body care and sexual behaviour, as well as a reduced appetite (Gonzales et al. 2008, Urton et al. 2005, Huzzey et al. 2007).

Acute clinical mastitis causing sickness in cows initiates a motivational conflict with respect to their behavioural priorities. Due to sickness, a cow will be motivated to lie down in order to rest and enhance the healing process by minimizing the consumption of body energy reserves (Johnson 2002, Weary et al. 2009). However, lying down may cause uncomfortable or painful feelings because of a painful udder, which can limit the lying time (Fogsgaard et al. 2012, Medrano-Galarza et al. 2012).

Hart (1988) considered sickness behaviour more as an expression of the animal's motivational state than a consequence of illness. Furthermore, Aubert (1999) concluded that the expression of certain behaviours in animals requires a hierarchical structure of motivational states that is influenced by different stimuli, which are continuously updated. Motivation is assumed to make an essential contribution to behaviours when an animal copes with different challenges, which in turn directly affects the welfare status of the animal. An animal's changed welfare status reflects a changed pathological status, affecting the behaviours linked to the cow's coping strategies and motivational hierarchy (Broom 2006). This has been demonstrated in previous studies by Aubert (1999), Konsman (2002) and Dantzer (2001), in which sickness behaviours were considered as motivational states, originating from reorganized priorities and affected by different stimuli.

If sickness behaviour is prevented, recovery from disease is poorer (Johnson 2002). Furthermore, fear-motivated behaviours are reported to override sickness-motivated behaviours (Aubert 1999). Sickness responses in animals exposed to painful challenges have been reported to affect behavioural patterns, for example, in rats, lambs and cows (Barrientos et al. 2009, Molony et al. 2002, Tom et al. 2002, Chapinal et al. 2010a and b), demonstrating that there is variation in an animal's sickness behaviour.

Some elements of sickness behaviour have been used to automatically detect disease outbreaks in cattle. Changes in feeding behaviour and feed intake in cattle have been used to predict ketosis (Gonzales et al. 2008), bovine respiratory disease in beef cattle (Sowell et al. 1998, Buhman et al. 2000), calf morbidity in feedlots (Quimby et al. 2001) and metritis in dairy cows (Urton et al. 2005, Huzzey et al. 2007). In addition, changes in sucking behaviour are useful for identifying sick unweaned calves (Svensson and Jensen 2007). Borderas et al. (2008) noted changes in the behavioural patterns of calves during lipopolysaccharide (LPS) challenge. Following the challenge, rumination, hay intake and self-grooming decreased. In addition, inactivity during lying and standing bouts increased (Fogsgaard et al. 2012). Experimentally induced mastitis has been reported to trigger sickness behaviour in cows (Fogsgaard et al. 2012) and affect cow behaviour and well-being. Moreover, not only are lying and feeding behaviour affected (Cyples et al. 2012), but changes in dry matter intake and milk yield have also been reported (Yeiser et al. 2012).

Knowledge of the consequences of mastitis for cow behaviour is still rather limited. Therefore, more precise information is needed on the type of behaviour and how it changes during mastitis, as well as whether it can be detected by observing a cow's behavioural patterns.

Cow behaviour and automatic milking

Automatic milking systems offer good possibilities to measure health-related parameters in cows, such as the milk yield, milk flow, milk quality parameters, cow activity and deviations in the milking process. A study by Miquel-Pacheco et al. (2014) confirmed that cows change their behaviour in an AMS in response to a decrease in a particular health status, which in their case was lameness.

Pastell et al. (2006) found that changes in milking behaviour provide valuable signals of leg or other health-related problems. They also noted that lame cows lift their legs more frequently and place less weight on a sore hoof during milking (Pastell and Kujala 2007). Borderas et al. (2004) reported that cows that regularly visit the milking robot walk better (less limping) than those that visit the milking robot less frequently. Furthermore, a higher kicking frequency during milking in cows may be a result of pain or discomfort caused, for instance, by teat lesions (Rousing et al. 2004).

Along with the rapid change towards precision livestock farming, the effectiveness of technology has become more prominent and its support for cow health and welfare. Data patterns obtained from AMS have been modelled to predict and detect clinical mastitis (Kamphuis et al. 2008 a and b). Moreover, Rasmussen et al. (2007) found that the frequency of insufficient milking increased from 5% to 30% one week before a mastitis outbreak, which indicates that mastitis can impact on the success of the milking process. In addition, the effectiveness and availability of automatic milking is affected by cow behaviour (Jacobs et al. 2012). However, more information is needed on the link between cow behaviour and milking success, and the frequency of incomplete milkings in automatic milking systems.

Technologies for health monitoring

Numerous technologies have been developed and tested for assessing the health status of animals, especially dairy cows. These have involved various novel sensors and technologies in data collection, concentrating on animal observations. Precision livestock farming, as described by Berckmans (2008), "consists of measuring variables on the animals, modelling these data to select information, and then using these models in real time for monitoring and control purposes." A recent review on the use of novel sensor technologies to support health management on dairy farms revealed that considerable effort has been put into finding effective technologies for detecting the most costly welfare issues, such as mastitis, fertility and locomotion problems (Rutten et al. 2013). For example, Polat et al. (2010) and Colak et al. (2008) reported that udder temperature correlates closely with a cow's rectal temperature, and that an infrared thermal camera can be used to detect changes in udder temperature caused by mastitis. Pastell et al. (2006, 2008) focused on leg health by measuring the weight distribution between legs during milking. Changes in the distribution of weight between the legs or changes in milking behaviour were found to be valuable signals of leg or other health-related problems due to the more frequent shifting of weight off and lifting of a sore hoof (Pastell & Kujala 2007). Moreover, vision-based systems have been applied in lameness detection (Song et al. 2008). Image analysis has been used to monitor locomotion and posture in cows (Cangar et al. 2008). Various devices exist to record activity in barns, locomotory activity during milking (Pastell et al. 2009, Chapinal et al. 2011), rumination and changes in milk quality (Hovinen and Pyörälä 2011).

III Aims of the study

The overall aim of the research reported in this thesis was to find new health- and behaviour-based indicators to help in establishing more efficient technological tools for dairy management in large herds. The more specific aims were to identify changes in cow behaviour and udder skin temperature in response to mastitis, as well to assess the applicability of a thermal infrared camera in detecting udder skin temperature changes in cows during mastitis. The objective was to determine how strongly mastitis affects the daily behavioural rhythm of cows and assess the sensitivity of novel tools, such as a thermal infrared camera, in detecting mastitis. Moreover, we examined cow–technology interactions and problems during milking by using video recording technology.

The main research questions

- Can a change from normal to sickness behaviour be identified in cows exposed to mastitis?
    - The hypothesis was that cows will change their normal behaviour pattern when sick. One such expected change was an increase in restlessness behaviour.
- Can a thermal infrared camera be used to detect changes in the udder skin temperature of cows during mastitis?
    - The hypothesis was that udder skin temperature of cows increases during mastitis, and that this increase can be detected with a thermal infrared camera.
- Can cow behaviour during milking be detected and described, and what are the main characteristics of cow–technology interaction causing deviations during automatic milking?
    - The hypothesis was that restless behaviour in cows during milking causes incomplete milkings and impairs the milking process.

Specific aims of the study

- To investigate how cow behaviour changes when exposed to mastitis (article I).
- To investigate the viability and application of novel technological tools to detect changes in cows (articles II and III).
- To study the effect of milking on cow behaviour and the effect of cow behaviour on the success of milking and problems occurring during the milking process (articles III and IV).

IV Materials and methods

The experimental protocols were approved by the Ethics Committee for experimental studies on animals of the University of Helsinki, Finland (experiment I, original articles I–II). For experiment II (original article III), animals were only video recorded. The Ethics Committee for the use of experimental animals at MTT Agrifood Research, Jokioinen, approved experiment III (original article IV).

Animals and housing

For the mastitis experiment (articles I–II), six cows were used as experimental animals (5 Finnish Ayrshires and 1 Holstein-Friesian); of these, five cows were in their first lactation and one cow in the second lactation. The cows were housed in a stanchion barn of 68 milking cows in tie stalls bedded with wood shavings. The cows had free access to good quality silage and water and were fed with concentrate six times daily according to their state of lactation. All cows were placed in the same row, beside each other. The cows were milked with a pipeline milking machine (DeLaval Harmony, DeLaval International AB, Tumba, Sweden) twice a day at 05:30 and 17:30. The lights were on between 05:00 and 20:00 and a dim night-light was provided in addition to the natural light coming from the windows.

The second experiment (article III) was carried out at the Suitia experimental farm of the University of Helsinki and the third experiment (article IV) was conducted at Haapajärvi Agricultural School Farm, Finland. Housing in experiments II (article III) and III (article IV) were mostly similar to each other. Cows were housed in a warm loose housing system with a single unit automatic milking system (VMS, DeLaval International Ab, Tumba, Sweden). The experimental group at Suitia experimental farm (article III) consisted of 38 Holstein-Friesian cows. Of these, 58% were primiparous, 24% in the second, 10% in the third and 8% in the fourth lactation. The cows had free access to good quality silage and water, as well as to concentrate feeders adjusted to feed them according to their state of lactation. The Haapajärvi Agricultural School Farm (article IV) had similar arrangements as Suitia. The experimental group consisted of 10 multiparous Ayrshire cows. In addition to an AMS, the Haapajärvi barn also included a herringbone parlour with three milking places (DeLaval international, Ab, Tumba, Sweden). Both milking systems in Haapajärvi were installed in the same loose-housing barn and under the same herd management. Forced cow traffic with separation gates in the parlour were used on both farms.

Measurements

Clinical measurements, sampling

For the mastitis experiment (articles I–II), the clinical examination of the cows and milk sampling of the experimental and control quarters are presented in Table 1.

Follow-up of clinical signs

Local udder signs were considered slightly changed if the udder was slightly swollen and sore and severely changed if it was painful, firm and severely swollen. Milk appearance was considered slightly changed if milk was slightly discoloured and/or contained small flakes. Severely changed milk was strongly discoloured or watery, and/or contained large clots.

Behavioural parameters

The definitions of the most relevant behaviours recorded during the research of this thesis appear in Table 2 (articles I–II), where the mean bout length, total daily duration and frequencies were determined for each of the behaviours. In addition, to examine the effects of time from the induction of mastitis, the data from the recordings were divided into two-hour periods. Cow body postures were scored as either standing or lying. To establish whether cows avoided lying on the affected udder quarter, the side on which the cows were lying was registered. In addition, we registered eating, drinking and ruminating of the cows. Specific observed behaviours during milking (articles III–IV) are presented in Table 3.

Table 1. Protocol for experimental mastitis in six cows induced with E. coli lipopolysaccharide.

	Time of the day							
Days	05.00	07.00	09.00	11.00	13.00	15.00	17.00	19.00
-1	A[1], B[2], Y[3]	NaCl[4]	A	A	A	A	A, Y	A
0	A, B, Y	LPS[5]	A	A	A	A	A, Y	A
1	A, B, Y		A	A	A	A	A, Y	A
2	A, B, Y		A	A	A	A	A, Y	A
3	A, B, Y		A	A	A	A	A, Y	A

[1] A = clinical examination of the cow, milk sampling and thermal imaging of experimental and control quarters
[2] B = bacterial sampling of milk
[3] Y = milk yield measurement
[4] NaCl = NaCl infusion of the experimental quarter
[5] LPS = *Escherichia coli* lipopolysaccharide infusion of the experimental quarter

Table 2. Ethogram for observations in the mastitis experiment

Behaviour/Posture	Description
Standing	Cow is standing on four legs
Lying down	Abdomen of cow touches the floor
Lying on the right/left udder	Right/left udder side is on the ground
Stepping	Cow lifts a leg (up, front, side, hind directions)
Eating concentrate	Cow has muzzle in the concentrate bucket or above it and shows chewing movements or licks concentrate from the floor under the bucket
Eating silage/hay	Cow has muzzle on the silage or shows chewing movements above the feed
Drinking water	Cow has muzzle in the water bowl
Rumination	The jaws move rhythmically from side to side, not related to eating.
Body care	Cow licks its body, or head moves rhythmically while muzzle touches any body part

Table 3. Ethogram for observations in the milking experiments

Behaviour	Description
Kick-off	Cow kicks the milking cluster off from the udder
Weight transfen	Cow lifts a leg and shifts her weight to the other leg
Stepping	Cow lifts a leg from the ground and puts it back down for a while
Kicking	Cow lifts a leg to make kicking movements

Measuring technologies

Video recordings

Cow behaviour was filmed analogically for 12-hour periods (articles I–III) or a digital camera was used to record milking during the two-week experimental period (article IV). The cameras were installed (articles I–II) above the cows on the barn ceiling. Two cameras recorded the behaviour of two cows from the rear and two cameras from the front. Twelve cameras in total were connected to a multiplexer and one VHS video recorder (Panasonic 6070). Three cameras (for article III) and one camera (Panasonic for article IV) were attached in front of the milking parlour on the wall and floor structure. The data were recorded with a dep-800 IDVR digital recorder (Dynamic Electronics and Protection, Espoo, Finland). The recording system consisted of a PC with a 4-channel video capture card, Skyview RX (Dynamic Electronics and Protection, Espoo, Finland) surveillance software and the Windows XP Professional operating system. Recordings were saved automatically on removable 120 gigabyte hard discs. Recording was set at five frames per second. Behaviours in each experiment were scored continuously with The Observer© (Noldus, the Netherlands) video analysis software.

We assessed the functioning of the AMS based on its success in performing the milking process without deviations. The milking process in the AMS was analysed from video recordings of 300 milkings observed to assess cow behaviour linked to AMS functioning and deviations (article III). The results were then compared with reports provided by the AMS computer programs. The reports comprised basic cow information and data on AMS deviations.

For the fourth article, a total of 200 milkings were observed. Cow behaviour and deviations during milking were recorded and subsequently scored continuously with the same method by using The Observer© (Noldus, the Netherlands) software. The first group of cows was brought to a separate waiting area in front of the herringbone milking (HRB) and automatic milking system (AMS) units. Heart rate measuring devices (Black box, Tampere University of Technology, 2006) were attached around the cows with flexible belts to record HR during milking.

Detecting udder skin temperature with a thermal camera

Thermal images were taken to monitor the change in the quarterly udder temperature of cows (articles I–II). Three consecutive images were taken at one-second intervals before clinical sampling at a distance of approximately 50 cm from each angle. The orientation for images was from the lateral and the medial angles from the experimental and control quarters, and from the craniolateral side of the experimental quarter. Images were taken at each sampling throughout the five-day experimental period with a handheld thermal camera (IR FlexCam Pro, Infrared solutions, USA).

The thermal camera used operated in the 8 to 14 µm spectral band. The thermal resolution of the camera was 0.09 oC and it was calibrated to the temperature range of 0–100 oC. The camera used microbolometer detectors and had a 160 x 120 pixel focal plane array. The camera had an internal recalibration feature, which automatically calibrated the detector to provide readings corrected for the ambient temperature. The emissivity value was set to 0.98, which is the value measured from human skin (Jones and Plassmann, 2002).

The maximum temperatures of the three images were averaged and recorded as the udder temperature for the sampling. The values from the lateral side were selected for use in the analysis, since they were found to most precisely show the temperature rise of the udder during the LPS challenge (Figure 1.).

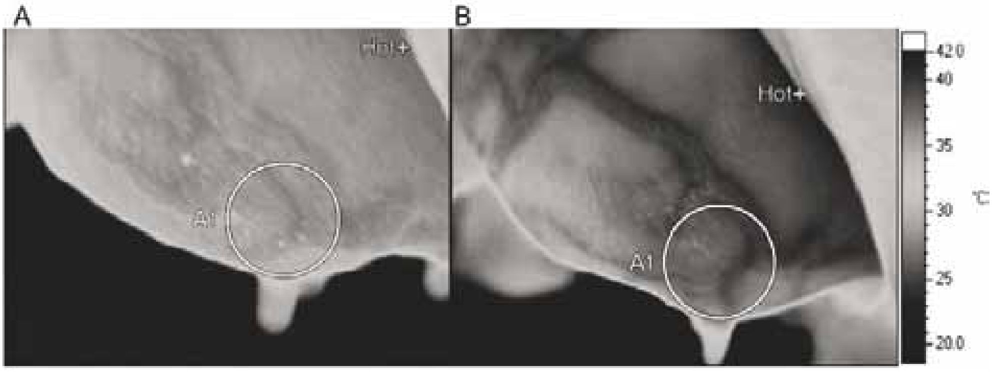

Figure 1. Thermal images of the lateral angle of the experimental udder quarter (left fore) a) before and b) during the induction of acute endotoxin mastitis. A1 = 40× 40 pixel area above the teat; Hot+ = position of the maximum udder skin temperature of the image.

Statistical analysis

To analyse the impact of acute endotoxin mastitis on cow behaviour (article I), a paired t-test was applied to study the effects of day (placebo vs. induction) on the daily durations, bout frequencies and bout durations of registered behaviours. To examine the effects of the time of day, two-hour means for the behaviours were calculated. The means were analysed with repeated mixed models. All the statistical analyses were conducted with SPSS 13.0 for Windows (SPSS Inc. Chicago, IL).

Inflammation indicators in milk and udder temperature were analysed with linear mixed models, taking repeated measures into account (article II). The fixed factors were hour (the time from the induction of mastitis), quarter (experimental or control), and the interaction between hour and quarter. The cow was introduced as a random effect. No covariates were used. Homogeneity of variances was evaluated with a scatter plot of residuals and predicted values. Pearson's correlation coefficient was calculated between the rectal temperature and udder temperature. All statistical analyses were performed with SPSS 13.0 for Windows (SPSS Inc., Chicago, IL).

Linear mixed models were used (articles III–IV) for the analysis of the effect of milking on cow behaviour. All the statistical analyses were conducted with SAS/MIXED software (version 9.1 SAS, 2004). This revealed that square-root transformation was needed for the analysis of kicks, weight transfers, steps and the somatic cell count. Respiration and heart rate data were not transformed. Statistical analysis was based on a crossover experimental design. The experiment had two periods, and two measurements were taken within each period: one during the morning milking and another during the evening milking. The milking time was used as a repeated factor in statistical analyses.

V Results

The most important results of the experiments appear in this section, which summarizes the findings from articles I–IV. For more detailed results, the reader may refer to the original papers included at the end of the thesis.

1. Can we identify a change between typical and sickness behaviour in cows exposed to mastitis? (Article I-II)

Clinical signs

All cows developed clinical mastitis, showing both systemic and local signs after the Escherichia coli lipopolysaccharide (LPS) challenge (Figure 2.). Rectal and udder temperatures increased from 4 to 8 h after the induction (post challenge, PC; P < 0.001) and the udder quarters became swollen. The udders of cows were visibly swollen from 2 to 4 h after the induction, and did not return to normal during the experimental period (5 days, 100 hours). Rectal temperatures of the cows started to rise from 4 to 6 h PC and remained above 39.2 °C from 6 to 10 h PC, reaching peak values at 6 h. Body temperatures returned to normal within 12 h PC (Figure 3.). Milk electrical conductivity and the somatic cell count (SCC) started to increase at 4 h PC and milk NAGase activity at 8 h PC, and remained higher than in the control quarters during the following 24 h.

All cows had mild to moderate systemic and local signs and changes in milk ap-

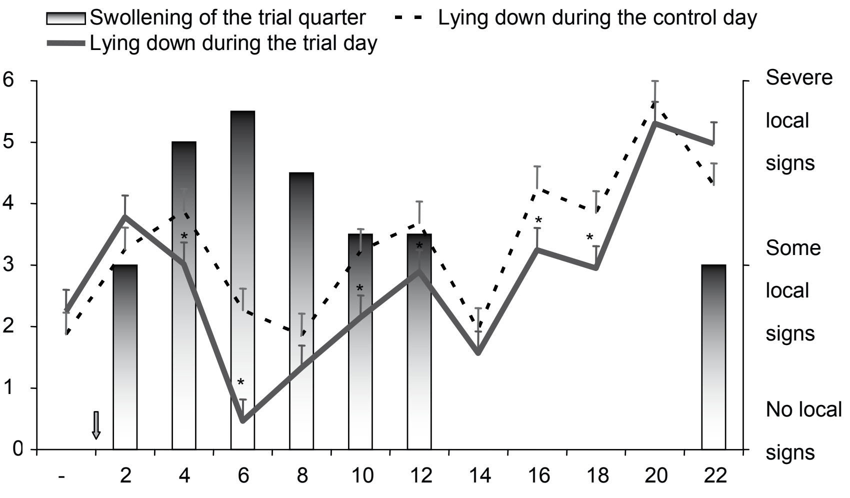

Figure 2. Local udder signs also started to appear in cows 4 hrs after challenge, and the time spent lying down decreased

pearance 2 h PC, with a few exceptions: changes in the milk appearance of one cow were seen 4 h PC, and local signs and changes in the milk appearance of another cow were seen 4 h PC. Both the rectal and udder skin temperatures and systemic signs remained above normal until from 8 to 10 h PC, as illustrated in Figure 3.

All quarters except 1 were swollen until the end of the experimental period, and milk appearance did not normalize during this period. Local signs in the udder were severe in five cows on the day of the challenge. Severe changes in milk appearance were visible for three cows in sporadic samplings. No local signs or changes in the milk appearance were detected in the control quarters. The average milk yield of the cows on d –1 and from the morning milking of d 0 was 13.0 ± 0.4 kg per milking, and from the evening milking of d 0 and at both milkings of d 1 was 11.5 ± 0.1 kg per milking (P = 0.50).

Changes in behaviour

On the day of acute endotoxin mastitis induction, compared with the control day before induction, cows exhibited more frequent stepping behaviour (P = 0.02), tended to spend less time lying and less time lying on the side of the affected udder quarter (P < 0.07, for both). The cows also tended to stand longer on the induction day than on the control day and the standing bouts were longer (P < 0.07). They spent a longer time eating silage during the induction day than the control day (P < 0.05) and they also elevated their stepping frequency.

Daily behavioural rhythm

Mastitis had an effect on the daily rhythm of the cows. Statistically significant (P < 0.05) interactions between the time of the day and day of the experiment were found for the mean hourly durations of lying, ruminating and drinking water, as illustrated in Figures 4., 5., 6 and Table 4.

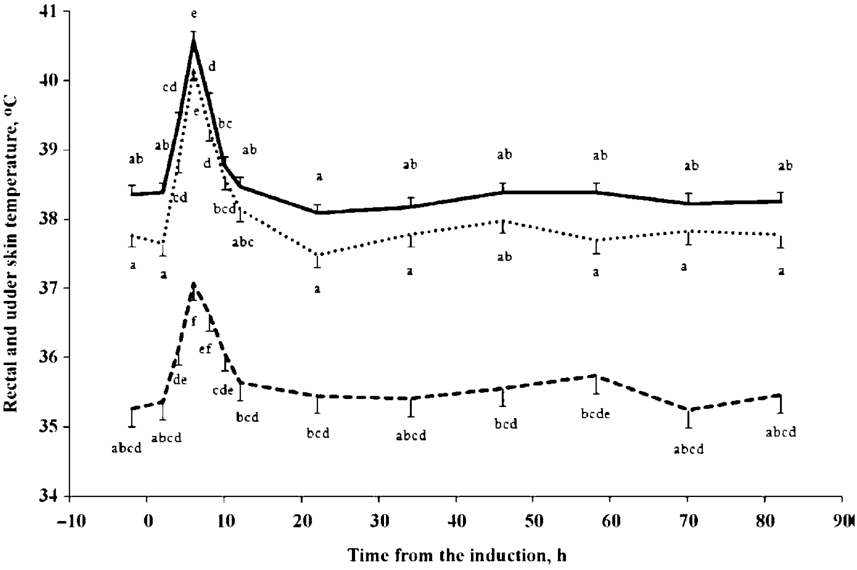

Figure 3. Rectal temperature (solid line), maximum udder skin temperature of the image (dotted line) and udder skin temperature of the 40 × 40 pixel area above the teat (dashed line) for the lateral angle of the experimental quarters of 6 cows throughout the experimental period, excluding d –1, x ± SE. Escherichia coli LPS was infused at time point 0. a–f Different letters indicate statistically significant differences between mean temperatures (P < 0.05) at different sampling times.

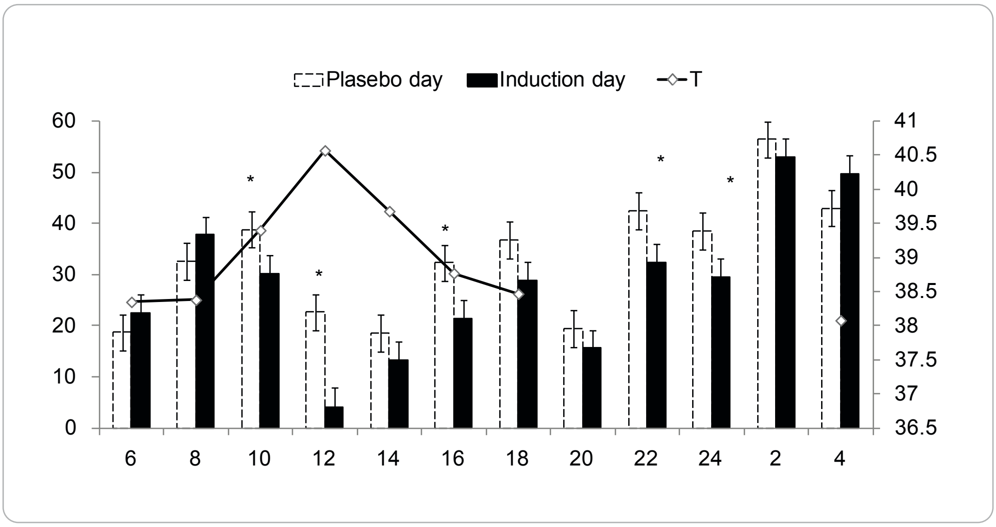

Figure 4. The impact of LPS mastitis challenge on six dairy cows; time spent resting and body temperature.

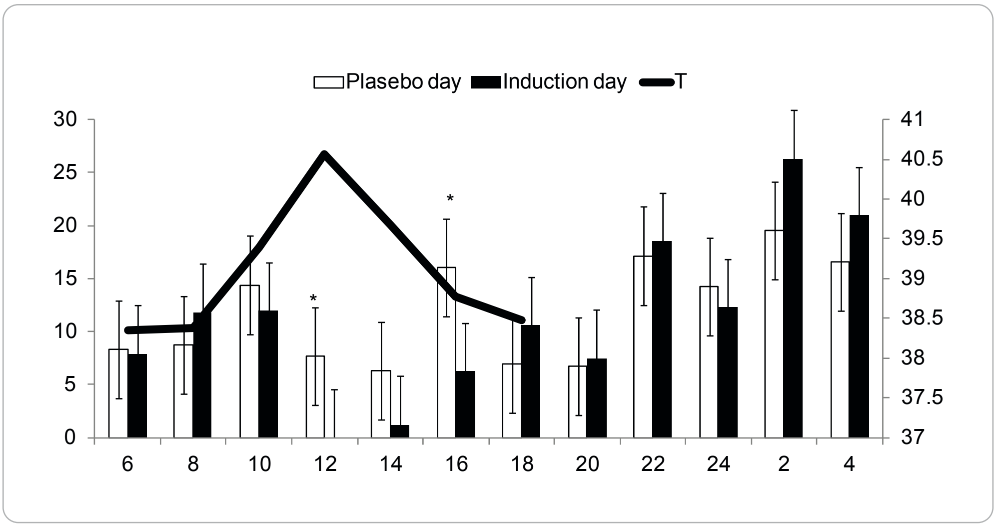

Figure 5. The impact of LPS mastitis challenge on six dairy cows; time spent ruminating and body temperature.

The mean time spent ruminating decreased between 4 and 8 h PC (at 12:00 and 16:00 PM) compared with the control day ($P < 0.05$). Cows also tended to reduce their drinking between 4 and 6 h PC (from 10:00 to 12:00 AM) compared with the control day ($P < 0.05$). Furthermore, cows increased their drinking at 10 h PC (at 16:00 PM) ($P < 0.05$).

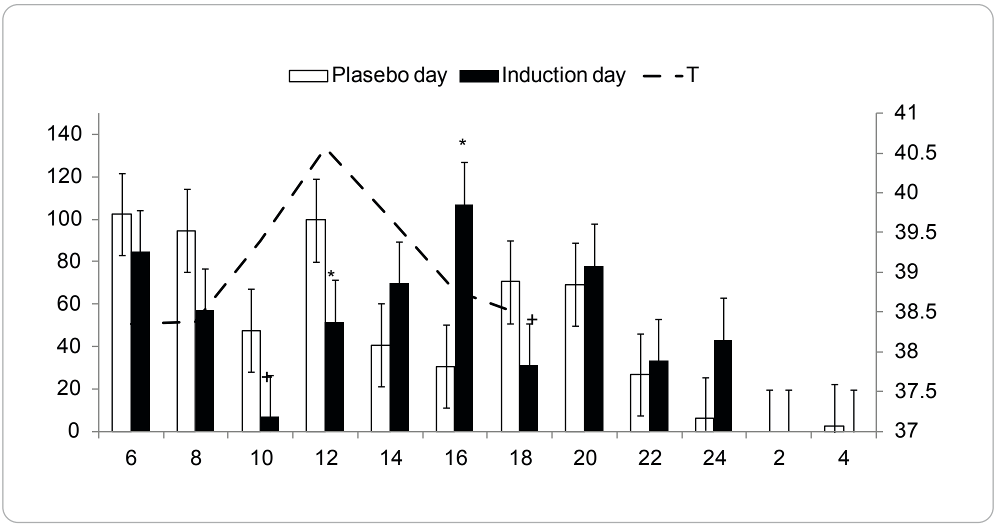

Figure 6. The impact of LPS mastitis challenge on six dairy cows; time spent drinking and body temperature.

Table 4. Resting behaviour of six dairy cows before (placebo day) and after induction of acute endotoxin mastitis (induction day). Results are presented as the daily mean and the standard error of paired differences (SE).

Behavioural variables	Frequency (No.)			Total daily duration (min)			Mean bout length (min)		
Day	Placebo Day	Induction	SE	Placebo Day	Induction	SE	Placebo Day	Induction	SE
Standing	23.33	21.0	2.19	638.61	759.59*	30.03	27.73	36.63†	3.71
Lying down	23.33	21.0	2.19	800.40	670.30*	30.08	34.88	32.59	3.46
Lying down on the control quarter (right)	10.33	8.5	1,64	381.30	326.04	29.33	38.28	40.94	5.56
Lying down on the induced quarter (left)	11.17	10.67	0.96	418.49	353.15†	28.43	37.61	33.76	3.62

†; P ≤ 0.07; *; P ≤ 0.05, se = standard error of mean difference

2. Can a thermal infrared camera be used in detecting temperature changes in the cows' udder during mastitis (Article II)?

Infrared thermal camera and body temperature

All cows developed mastitis after the Escherichia coli lipopolysaccharide (LPS) challenge. The mean temperature of the udder and control quarters was elevated 4 h post-challenge ($P < 0.01$). However, the udder temperature of the cows did not increase before an increase was recorded in the rectal temperature. The correlation between the rectal temperature and mean udder temperature for the lateral angle of the drawn circle was $r = 0.92$ ($P < 0.001$), and the correlation between the rectal temperature and maximum udder temperature for the lateral angle of the image was $r = 0.98$ ($P < 0.001$).

The maximum udder temperature (TU) of the image and the mean TU of the measured area increased in parallel for both angles of the experimental and control quarters. At 6 h PC, 5 of the 6 cows had a clearly increased mean TU in the measured area. The udder temperature decreased to normal levels within 8 to 10 hours after the challenge, in parallel with or a slightly later than the rectal temperature.

3. Changes and main characteristics of cow behaviour during milking (Articles III–IV)

Changes in milk

We found no statistically significant difference between milking systems (automatic milking system, AMS, and herringbone system, HRB) in the milk somatic cell count (article IV). When milked in the AMS and the HRB, respectively, the average somatic milk cell count was 45.9 cells/µl and 51.1 cells/µl. The cell count varied between 16.1 and 114.2 cells/µl. However, a clear difference in the milk cell count was detected between morning and evening milkings, with higher milk cell counts in the morning ($F = 17.2$, $P < 0.001$). The change in milking management caused no marked peaks in the cell count.

Behavioural changes following a change between herringbone (HRB) and automatic milking systems (AMS)

A change between the AMS and HRB did not cause marked changes in cow behaviour (article IV). Independent of the milking system, cows expressed greater behavioural activity, as measured by weight transfer, in morning than evening milkings ($F = 8.21$, $P < 0.05$). On average, cows exhibited 2.2 weight transfers per milking in the AMS and 2.6 in the HRB.

An interaction effect between the type and time of milking was detected in bovine stepping behaviour. In the AMS, cows stepped more often during the morning than the evening milking (21.2 vs. 16.1 steps, $F = 6.02$, $P < 0.05$). Kicking behaviour did not differ according to the time of milking or the type of milking system. Kicking varied on average between 1.3 and 1.8 kicks during the morning milking and between 1.6 and 2.0 kicks during evening milking.

Kick-offs, i.e. kicking the milking device completely off the udder, were also recorded, but the total number remained too low for statistical analysis. In the HRB, 16 kick-off cases occurred during the experimental period; the corresponding number in the AMS was 9.

The health records of cows were checked during and after the experiment. No changes in health due to the experiment were detected.

Cow behaviour and deviations in automatic milking

Six of the 300 milkings (2%) were not assessed due to poor visibility of the milking process or the stockperson standing in front of the camera while treating the cow.

The number of milkings in the automatic milking system (AMS) per cow during the 3-day observation period varied from 5 to 10. The milking interval of the group averaged 8 h 55 min (SD 0.07), ranging from 6 h 46 min to 13 h 2 min. Of the 300 milkings, 247 (82%) were completed successfully. The types of deviations are presented in Figures 7 and 8.

Although failures causing incomplete milkings occurred, in most cases the machine was able to compensate the procedure such that all phases of the milking were properly performed. If the AMS was

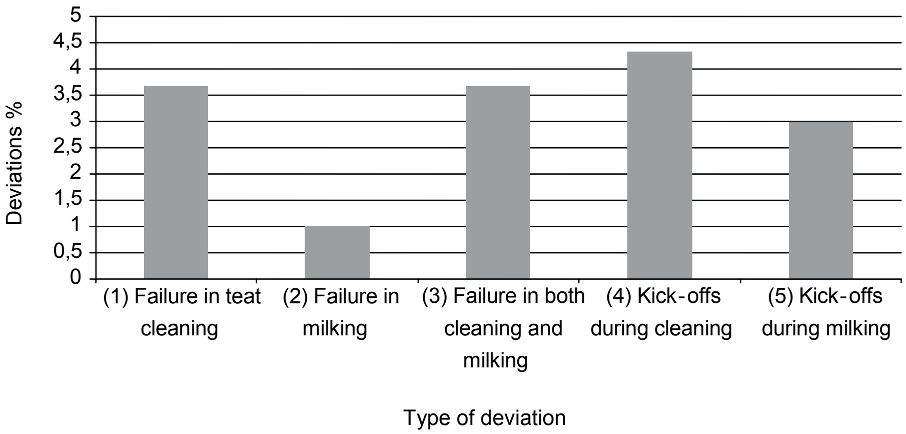

Figure 7. Types of deviations during automatic milking (in 300 observed milkings).

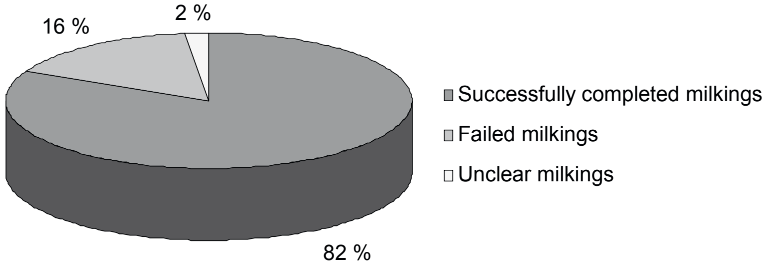

Figure 8. Types of deviations during automatic milking (in 300 observed milkings).

able to compensate for failures and the cow was milked, the milking was recorded as successful in the results. However, a total of 47 (16%) incomplete milkings were observed. Failures occurred in either the teat cleaning or milking processes, or in both.

Two unclear cases occurred because a cow moved continuously such that the observer could not be sure of what was happening. In the teat cleaning process, 11 (4%) deviations occurred. In the milking process, milking was incomplete in only 3 (1%) cases. However, 11 (4%) cases were deficient in both teat cleaning and milking.

Kick-offs were detected in 31 of 294 milkings, with 14 of them occurring during teat cleaning; 1 (0.3%) of them was compensated by the machine, such that the procedure was recorded as successful, and 13 (4%) were uncompensated. During milking, 17 kick-offs were detected; 8 (2.7%) of them were compensated and 9

(3%) were uncompensated. Only 29% of all deviations during the teat cleaning and milking processes were successfully compensated by the robot.

Observed kick-offs were compared with kick-offs in the AMS computer report. The latter consisted of normal kick-offs, i.e. the milking unit being kicked of by the cow, a teat cup dropping on the floor when the teat was empty, and a cow standing on the milking pipe, resulting in detachment of the teat cup. Some teat cups also fell when the robot gripper tried to attach the teat cup to another teat. All of these situations were registered as kick-offs in the AMS software.

Origin of failures in automatic milking

Deviations during milking were of two types: cows disturbed the milking process in 55% of the failures, and failures originating from the AMS were detected in 45% of the failed cases, which are shown more precisely in Table 5.

Problem cows during automatic milking

Six problem cows out of 38 were clearly identified. Three of these problem cows caused 52% of all the failures in teat cleaning, milking, or both. These six problem cows caused the most important deviations in the milking process. After a failed milking of these six cows, the stockperson had to complete the milking procedure manually on each observation day.

The most severe problems were detected with two cows that had no successful unaided milkings during the 3-day observation period. The first problem cow had three unsuccessful milkings that were not manually assisted and two successful manually assisted milkings during the observation period. The second problem cow made seven milking attempts, but none of them were successful. The stockperson had to put the milking robot into manual mode to assist the robot in milking the cow.

Four of the six problem cows experienced milder problems in the milking process.

Table 5. The origin of failures during milking were caused both by cow behaviour and AMS functioning.

Failures caused by cow	Number	Proportion, %
1) Kick-offs during washing (uncompensated)	13	28%
2) Kick-offs during milking (uncompensated)	9	19%
3) Cow caused failures in teat washing	1	2%
4) Cow caused failures in milking	1	2%
5) Cow caused failures in both washing and milking	2	4%
Sub-total	26	55%
Failures caused by AMS		
1) Failures in teat washing	10	21%
2) Failures in milking	2	4%
3) Failures in both washing and milking	9	19%
Sub-total	21	45%
Total	**47**	

Individual failures in the milking process varied from 40% to 83% for these four cows. Their failures comprised incomplete teat cleaning, milking or uncompensated kick-offs. Restless behaviour, i.e. kicking the teat-washing device and detaching teat cups, had a marked impact on their milking procedure. Problems during milking became progressively more serious if immediate action by the stockperson was not taken to correct the situation.

The status of these six problem cows was rechecked six months after the experiment. The situation had not improved, and two of the cows were culled. The reason for culling the first problem cow was her character and the problem behaviour exhibited in the AMS. The cow could not become accustomed to the milking procedure, continuing to kick the machine, resulting in failed milkings. The second problem cow was culled because of a continuously high somatic cell count. Of the four remaining problem cows, three had been treated several times for fertility and reproduction problems. One cow had no health problems.

VI Discussion

Cows in the studies presented in this thesis changed their behaviour after the induction of mastitis. However, a change of milking technologies did not impact on cow behaviour. When the health status of the cows deteriorated, changes were detected in both their behavioural pattern and clinical status. At present, sick cows are often detected in the late stages of infection when their milking interval is already prolonged (Miquel-Pacheco et al. 2014). Our results support the view that automated observation tools providing combined information on cow behaviour, clinical status and milk appearance would best serve in detecting early warning signs of a change in health status.

1. Can we identify a change between typical and sickness behaviour in cows exposed to mastitis? (Articles I–II)

Cows suffering from endotoxin-induced acute mastitis showed some typical features of sickness behaviour, e.g. a poorer appetite. In contrast to typical sickness behaviour, cows did not spend more time lying, but stood more, and avoided lying on the side of the sick quarter. Moreover, in contrast to earlier findings describing increased lying and lethargy, cows exhibited increased stepping. Cows increased their standing time simultaneously with the local swelling of the udder quarter and an elevated temperature. The most promising health indicators were identified in lying, ruminating and drinking behaviour patterns. Moreover, changes in cow behaviour occurred rather concurrently with clinical changes, with changes in udder swelling becoming visible between 2 to 4 hours post-challenge.

Change in behavioural priorities and patterns

Mastitis was found to have consequences for both cow behaviour and physiology that made the cows change their behavioural pattern. Due to sickness, cows reorganized their behavioural priorities and partly shifted their normal behaviour towards sickness behaviours. Mastitis caused changes in behavioural patterns, such as decreased lying time and changes in ruminating, as well as changes in the clinical health status. These findings are consistent with those of by Cyples et al. (2012), who noted that cows reduced their lying time during the first 20 h following a mastitis outbreak, when the most severe signs of experimentally induced mastitis were present. They reported similar indications of the potential of lying time as a valuable cow health indicator. Fogsgaard et al. (2012) also detected similar changes in cows, reporting increased standing and decreased lying during mastitis. Changes in clinical status were additionally visible, such as udder swelling and modified milk composition. However, the findings in this study contrasted with the previous results of Johnson (2002) and Weary et al. (2009), who described an increase in lying time. Furthermore, Weary et al. (2009) suggested that due to sickness, cows were motivated to lie down in order to rest and enhance the healing process by minimizing the consumption of body energy reserves.

In the present study, cows appeared to compromise their need for lying in order to avoid pain in the affected quarter of the udder, as also found by Bolles and Fanselow (1980). Since some of the behavioural changes in our study were opposite to those expected in sick animals, we suggest that pain experienced in the udder overrode the motivational state of the cows to express sickness behaviour, as also suggested by Aubert (1999). The changes in the behaviour of the cows with acute mastitis can be interpreted to reflect changes in behavioural priorities (Johnson 2002). We suggest that the cows started to reduce their lying time after the induction of mastitis, accompanied by a rise in the body temperature, but also along with the increasing local signs of udder swelling.

Increased vigilance

Cows in the present study showed more restlessness behaviours, a decreased lying time and increased preference for lying on one side, in line with the findings of Medrano-Galarza et al. (2012). It is also possible that discomfort caused by mastitis prevented the cows from lying and shifted their behaviour towards increased stepping to temporarily reduce their physical discomfort, as suggested by Cooper et al. (2007). Increased standing and stepping may, however, be a sign of increased vigilance, which helps the cows to detect a given stimulus at a given time (Dimond and Lazarus 1974). Increased vigilance has also been observed in lactating mice during an LPS challenge (Aubert et al. 1997). Moreover, this finding may originate from a different motivational hierarchy of cows in favour of the preservation of survival instincts, keeping the cows in a more vigilant state (Aubert 1999).

We found that cows increased their stepping behaviour when suffering from acute mastitis. The increase in stepping activity may have originated from the innate need of the cows to avoid or escape the uncomfortable experience, and may represent pain behaviour, as suggested by Molony and Kent (1997) and Chapinal et al. (2011).

Daily rhythm

Mastitis affected the daily rhythm of the cows. Significant interactions between the time of the day and day of the experiment were found for the mean hourly durations of lying, ruminating and drinking water. The mean time spent ruminating decreased between 4 and 8 h PC compared with the control day. Cows also tended to reduce their drinking between 4 and 6 h PC compared with the control day. Moreover, cows compensated their drinking at 10 h PC. Numerous findings confirm that lying down and rising may cause uncomfortable or painful feelings, which can change the locomotory activities of the cows, i.e. prohibit or prolong lying down movements (Niss et al. 2009, Galindo & Broom 2002, Juarez et al. 2003, Walker et al. 2008).

The present results are consistent with findings on sickness behaviours in cows during oligofructose overload challenge reported by Niss et al. (2009), and during ruminal acidosis challenge reported by DeVries et al. (2009). Lying time during sickness has been found to be a changing variable in cows (Fogsgaard et al. 2012). Previous studies have demonstrated that cows have a strong motivation to lie down (Jensen et al. 2005, Munksgaard et al. 2005), but they avoid doing so if the lying surface is uncomfortable (Haley et al. 2001). In this study, standing behaviour changed, as cows tended to stand longer on the induction day than on the control day and the standing bouts were longer. The extended standing after LPS challenge contrasts with the behaviour reported in calves, which tended to lie down after LPS challenge (Borderas et al. 2008). The motivational priorities of calves and their ability to cope with acute illness such as LPS challenge can differ from those of dairy cows. Moreover, different experimental settings may also explain this difference, as the calves were challenged intraperitoneally, while cows in the present study were infused via the teat. In addition, changes in the somatic cell count and NAGAse remained changed until the end of the induction day. This may be because unpleasant feelings preventing the cows from lying down, but changes in milk are not the best indicators of pain in cows.

Eating and rumination

We found that cows spent a longer time eating silage during the induction day compared with the control day. Ruminating behaviours are related to feeding and lying behaviour patterns (Schirmann et al. 2012) such that diurnal patterns exist for both ruminating and feeding and lying when observing cows for a longer period. However, no correlation was observed in our experiment between daily rumination times and daily lying time. In our study, cows adjusted their eating behaviours during mastitis by spending more time eating, especially eating silage. The cows were probably not eating more, but rather more slowly, due to fever-induced lethargy. Fitzpatrick et al. (2013) noted that cows ruminated less in the hours following LPS infusion and compensated for rumination later during the induction day, as was also the case in our study.

Cows spent proportionally more time ruminating while lying down. We suggest that cows adapted to a shortened lying time by increasing the proportional time spent ruminating. The increased rumination time has been explained by Schirmann et al. (2012), who reported a positive correlation between rumination time and lying time, indicating that periods of rumination are more frequent when cows are lying down.

To conclude, we suggest that a change in the lying pattern can serve as a valuable health indicator when observing behavioural changes related to mastitis, but is not sensitive enough. Thus, additional indicators are needed for effective health observation.

2. Can a thermal infrared camera be used to detect temperature changes in the cows' udder during mastitis (Article II)?

Thermal imaging was found to be a viable tool to detect changes in the udder skin temperature of cows during mastitis. As expected, the udder skin temperature rose simultaneously with the rectal temperature. Moreover, a slightly unexpected observation was that the elevated udder skin temperature was detected 4 h PC, while mild systemic signs, local signs in the udder and changes in milk appearance were already detected 2 h PC. Furthermore, automated tools for detecting changes in both udder skin temperature and modelling visual changes in udder appearance would enable earlier warning of a changing health status in cows.

Infrared thermal imaging was a viable and non-invasive tool in detecting the rise in the udder skin temperature during mastitis. Thermal images revealed that the mean udder temperature of the experimental and control quarters was increased 4 h PC. The udder temperature rose simultaneously with a rise in the rectal temperature. The maximum udder temperature measured by a thermal infrared camera increased in parallel for both angles of the experimental and control quarters. At 6 h PC, 5 of the 6 cows had a clearly increased mean udder temperature in the measured area. The thermal infrared camera was able to produce reliable information that was consistent with experiments reported by Colak et al. (2008), confirming that infrared thermography is sensitive enough to detect differences in udder skin temperature tested against the California Mastitis Test. Moreover, studies by Polat et al. (2010) confirmed our finding that an infrared thermal camera is sensitive enough to detect subclinical mastitis measured by observing changes in the udder surface temperature. Thermal infrared cameras have also been used in mastitis detection in sheep by Saraiva Martins et al. (2013). Their studies confirmed that the accuracy of thermal infrared cameras was sufficient to detect subclinical mastitis.

To conclude, our study confirmed that infrared thermal cameras can be used to detect changes in udder temperature caused by mastitis. However, when designing novel technological skin temperature-based tools for health detection, more research and development is needed to design farm-level applications to be integrated into existing technologies, for example in automatic milking systems.

3. Changes in and main characteristics of cow behaviour during milking (Articles III–IV)

We found that cows easily adapt to transfer between herringbone and automatic milking systems. However, some cows appeared to have difficulties in being properly milked in an automatic milking system. The underlying reasons for these difficulties originated from both the cows and the milking system. We found that the milking process should be regularly observed to ensure an optimal and effectively working human–animal–technology interaction, which is essential in dairy management. Behavioural activities and deviations during milking can be detected by using web-based video recording technology. Deviations in the milkings and causes of failures at milking are easy to analyse from recordings in order to ensure that cows are properly and regularly milked.

Factors impacting on the success of automatic milking

The overall success of the automatic milking process was on a reasonably high level (82%) in our experiment. Failures in milking originated from cow- and machine-based failures. This was in line with Hamann et al. (2004), who concluded that the occurrence of incomplete milkings was conditioned by both technical and cow-related circumstances. In some cases in our study, the milking robot apparently did not find a teat to be milked because the cows moved or kicked excessively. Furthermore, the milking interval of a single udder quarter or even the whole udder may have been prolonged if cows needed to queue in front of an AMS and the waiting time for milking exceeded their normal milking rhythm. Long milking intervals impair tight junctions in the cow udder, causing an influx of somatic cells into the milk. This influx of neutrophils has been found to continue even after cows were returned from once-a-day to twice-a-day milking (Stelwagen and Lacy-Hulbert 1996). In our study, this may have caused more pressure on the udder and changed the udder size, resulting in subsequent difficulties of the milking unit in attaching a teat cup to a teat. Hovinen et al. (2005) also found that an abnormal teat or udder structure in general caused, for example, unsuccessful teat cleaning. Moreover, the milking frequency in automatic milking systems should be modified according to the cow's state of lactation (Hovinen and Pyörälä 2011).

The milking of problem cows should regularly be checked to ensure that all udder quarters are milked completely. In addition, the deviation report that an AMS automatically produces is a valuable tool to identify potential problems in milking.

Cow behaviour in changing milking systems

We found that cows were able to cope with a change between milking systems and did not alter their behaviour when the milking system was changed within the same barn. However, cows displayed more behavioural activity, as measured by weight transfer between the legs, in morning than evening milkings. The difference in weight transfer was similar for the two milking systems.

An interaction effect between the type and time of milking was detected in stepping behaviour. When cows were milked in the AMS, they exhibited more stepping during the morning than in the AMS during evening milking or in the HRB during both the morning and evening milkings. Kicking behaviour did not differ in time or according to the type of milking system. We hypothesised that changes in the milking procedure might disturb the daily life of cows, which might be reflected in a change in their milking behaviour. This was not the case in our study. It has to be remembered that in our study, both milking systems were situated inside the same barn, and thus the social and physical environment of the cows remained the same. Cows in loose housing, and especially with automatic milking systems, develop certain behavioural patterns such as the milking interval, synchrony in the feeding time and resting behaviours (Rousing et al. 2006, Borderas et al. 2008). DeVries et al. (2011) found that standing and lying patterns in cows are especially affected by the milking frequency and milk yield.

Management of automatic milking

We found that cows having difficulties in being properly milked were dependent on the activity of the stockpersons to ensure complete milking. These findings are supported by Hovinen and Pyörälä (2011), who described the actions of stockpersons as being the most significant external factor in management affecting the udder health of a cow. Moreover, they found that proper milking management in automatic milking affects the udder health, cleanliness and milk yield of cows.

In addition, cow traffic in AMS barns has an impact on cow behaviours, as separation gates may affect milking, resting and feeding frequencies. DeVries et al. (2011) and Munksgaard et al. (2011) also observed that cow behaviour in an AMS is affected by daily care routines, such as fetching cows to be milked or placing feed in front of the cows. We suggest that cows were able to cope with the change in the milking process because the change was properly managed. Cows are adaptive individuals and exposed to various changes during their life on a farm. The importance of management routines and maintenance decisions, especially with cows having more important problems in adjusting to AMS, should be taken seriously. Rasmussen et al. (2007) found the frequency of unsuccessful milkings to increase from 5% to 30% one week before clinical mastitis occurred. This confirms that it is essential to observe the success rate of milkings and be able to take actions immediately after failures occur during milking. If the stockperson does not react in time to problems occurring during automatic milking, more problems are likely to occur and lead to deterioration in the welfare of the cows. Our findings emphasise the importance of an effectively working human–animal–technology relationship to secure proper and complete milking for cows in AMS (III), and are in line with those reported by Deming et al. (2013), who suggested that the success of an AMS system is largely affected by the combination of housing, management and cow characteristics.

VII Conclusions

The research reported in this thesis focused on the use of behavioural observations as an indicator of management changes (e.g. the milking system) and health problems (i.e. mastitis). Furthermore, technology (i.e. an infrared camera) could help to improve the accuracy of behavioural observations and improve the early detection of specific health problems. Our results improved knowledge of how a cow modifies its behaviour when developing mastitis. We found that detecting changes in behavioural patterns and assisting clinical detection with a thermal infrared camera measuring udder skin temperature (II) can serve as an additional tool for clinical mastitis detection (I). Furthermore, our findings on cow behaviour confirm that cows are able to cope with changes in management if they are properly managed and do not disturb their routines to a large extent. Moreover, we found evidence that half of the deviations occurring during the automatic milking process originated from cow behaviour, such as a cow kicking, lifting the legs and moving during milking (III).

When exposed to mastitis, cows also alter their behaviour during milking. As milking in automatic systems is carried out automatically by machines, the stockperson does not have information on the changing behaviour of cows during the milking process. Thus, there might exist an interplay between mastitis, the success of the milking process and cow behaviour.

1. Can we identify the change between typical and sickness behaviour in cows exposed to mastitis (Articles I-II)?

We found that behavioural changes in cows due to mastitis could be identified. The most interesting changes were detected in lying, eating and stepping behaviour during the bacterial LPS challenge. It was shown that inflammation simultaneously affected the health status and changed the behavioural priorities of cows. In contrast to our expectations, visual signs in the udder and changes in milk composition occurred only 2 h PC, while clinical and behavioural changes appeared at 4 h PC. However, the changes in lying and restless behaviours appear to be promising indicators for detecting signs in cows exposed to mastitis. We found that cows were more restless during the morning compared with the evening milking, suggesting that both individual and time-dependant behavioural variations need to be taken into account when considering restless behaviour in cows as a welfare indicator. The present study also yielded new information on cow behaviours during automatic milking that could be used when further developing cow observation methods in this system.

2. Can a thermal infrared camera be used to detect temperature changes in the cows' udder during mastitis (Article II)?

The transient increase in body temperature following experimentally induced clinical mastitis was successfully detected with the help of a thermal camera. The udder skin temperature rose simultaneously with the rectal temperature. However, local inflammatory changes of the udder, appearing earlier than the rectal temperature increase, were not detected by the thermal infrared camera. The capacity of thermal imaging to detect naturally occurring clinical mastitis needs to be examined under field conditions, where the systemic inflammatory reaction may be less pronounced in a longer process. This study demonstrated, however, that the udder is a sensitive site for the detection of diseases of dairy cattle causing a rise in temperature by infrared thermal camera. Thermal infrared imagining is a promising method for obtaining information on changes in the health status of cows. However, the changes in udder temperature did not serve as an early warning indicator, since visible changes in the udder and milk appeared first. Therefore, technologies based on visual modelling might further improve the timing of mastitis detection. To conclude, there remains a need to further develop more effective technologies to be integrated into barn conditions.

3. Changes and main characteristics causing deviations during automatic milking (Articles III–IV)

Half of the problems during milking were caused by the cows, meaning that the milking robot itself works at a satisfactory level. This demonstrates the importance of monitoring cow behaviour as well as incomplete milkings in the automatic milking system. The web-based video recording technology we used proved to be highly useful in observing cow behaviour, especially problem behaviour during the milking process. We suggest that the system can be used as a supplementary tool for observing the behaviour of animals in the barn as well as during procedures such as the automatic milking process. These tools provide additional information on the effectiveness of automatic milking, the success of teat cleaning and milking as well as deviations occurring during milking. However, further development in automating the observation system is needed to register and combine both the changes in cow behaviour and failures produced by the milking machine to provide a valuable support tool for herd management.

Our findings concerning cow behaviour when transferring between milking systems confirmed that cows, as adaptive individuals, do not show changes in behaviour during milking, even though their social synchronisation changed in connection with a change in milking system. Cows appear to be flexible individuals and cope well with properly managed changes in daily routines. The cost of changes in herd management in terms of cow health and welfare are often difficult to determine, since many factors cause variability, such as the impact of a different production environment, the management systems and also humans. In our study, these other factors were controlled, and the only parameter that changed for the cows was the milking procedure.

Cows react to their changing health status by altering their behaviour. This can be detected both by using technological tools and by observations of the stockperson, especially during milking. Moreover, the importance of the stockperson still remains in automatic milking to observe cows and the success of milking. I suggest that the daily working hours of the stockperson should be focused on observing and ensuring the functioning of human–animal–technology interaction in dairy management, as illustrated in Figure 9.

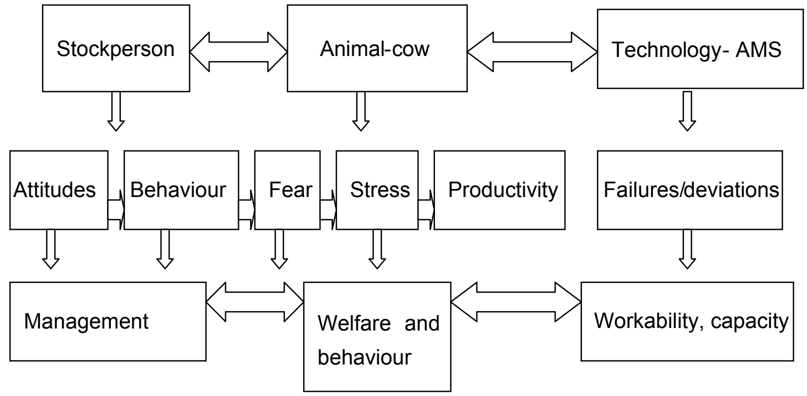

Figure 9. Complexity of the interaction between humans and animals (Kaihilahti et al. 2007, modified from Hemsworth & Coleman 1998) and technology, and how the workability of an automatic milking system impacts on the capacity of the system, the welfare status of the cows and management decisions.

4. Future implications for welfare studies

Questions requiring further studies

- Cows do change their behaviour in milking when exposed to mastitis. Therefore, the success of the milking procedure itself should be regularly observed. The role of the stockperson is even more important in large herds, and especially when automatic milking is used to observe cows and the milking process (Hovinen and Pyörälä 2011). This can only be done by ensuring a well-functioning human-animal-technology interaction in herd management. Therefore, the important interplay between behaviour, mastitis, milking and the human-animal-technology interaction requires further study.

- What is the cost of a change in motivational priorities for cows during the recovery process?

- What combined parameters are optimal for the early detection of acute disease outbreaks in terms of the health status of cows?

- What are the optimal combinations of detecting and modelling behavioural, physiological and clinical changes in cow health and welfare on a long-term basis?

- How can a well-functioning human-animal-technology relationship be ensured, especially when introducing new technologies into a herd?

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