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Validation of hock lesions as welfare indicator in dairy cows:

A macroscopic, thermographic and histological study

Master´s thesis

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1 Introduction

Hock lesions are one of the most common injuries in housed dairy cows and their prevalence is generally reported as high (Weary & Taszkun 2000, Keil et al. 2006, Fulwider et al. 2007, Rutherford et al. 2008, Kielland et al. 2009, Nuss & Weidmann 2013). The term hock lesion stands for various clinical presentations of hock damages, including the three major appearances hair loss, ulceration and swelling (Regula et al. 2004, Rutherford et al. 2008, Kielland et al. 2009, Laven & Livesey 2011, Potterton et al. 2011a, Brenninkmeyer et al. 2013, Burow et al. 2013, Lim et al. 2015). It is assumed that hock lesions originate from the animal lying on abrasive surfaces, sustained high local pressure or friction of the hard surface or collision of the hock with cubicle fittings (Keil et al. 2006, Brenninkmeyer et al. 2013, Kester et al. 2014). The relation between housing design and other animal- and management-related risk factors for hock lesions seems to be complex and interconnected (Kester et al. 2014). However, changes in cubicle characteristics, bedding material and access to pasture may decrease the prevalence of hock damages (Kielland et al. 2009, Brenninkmeyer et al. 2013, Nuss & Weidmann 2013, Kester et al. 2014). Injuries related to the housing systems often develop around the tarsal joint (Rutherford et al. 2008, Kielland et al. 2009) and it is assumed that especially severe injuries may result in pain and suffering (Wechsler et al. 2000, Gleerup et al. 2015). In account of this, the prevalence of hock injuries may be a fair indication of physical conflicts between the animal and the housing system and may display the degree of discomfort experienced by the animal (Rouha-Mülleder et al. 2010, Barrientos et al. 2013, Brenninkmeyer et al. 2013, Zaffino Heyerhoff et al. 2014). Furthermore, a positive correlation between hock lesions and other types of injuries and disorders has been reported (Fulwider et al. 2007). Hence, reducing hock lesion may also reduce lameness and may improve dairy cows health and welfare (Whay et al. 2003, Kielland et al. 2009, Brenninkmeyer et al. 2013). However, the specific impact of different types of hock lesions remains unclear, not least because of the copious quantities of different scoring systems across studies (Laven and Livesey 2011) and a lack of information about the severity of hock lesions and their impact on welfare (Lim et al. 2015).

2 Objective and hypothesis

The objective of this study was a comprehensive validation of the severity of hock lesions in lactating dairy cows. For this purpose, we investigated the degree of hair loss as well as ulcerations at both the hock and the point of the hock in terms of skin temperature and histological features of the skin. We hypothesized that already mild hair loss is relevant to the animals' health and welfare as regards inflammatory responses.

3 Literature review

3.1 The skin – the largest organ of a mammal

The skin (*cutis*) is the largest organ of a mammal and acts as a protective barrier against pathogens, toxins and trauma (Welsch & Deller 2010). It has an important role in fluid homeostasis and thermoregulation and functions as a sensory organ (Rosique et al. 2015). The *cutis* consists of two primary parts: the epidermis and the dermis (Fig. 1) (König & Liebich 2005). The epidermis is a stratified squamous epithelium that mainly consists of several layers of keratinocytes (Welsch & Deller 2010, Pastar et al. 2014). Keratinocytes are the predominant cell type in the epidermis and their function is the formation of a physical barrier against the environment (Welsch & Deller 2010). Keratinocytes originate from epidermal stem cells that are located in the basal layer (*stratum basale*) of the epidermis. During their differentiation keratinocytes produce more and more keratin and move towards the surface of the epidermis. They are passing the layers *stratum spinosum*, *stratum granulosum* and *stratum lucidum* (König & Liebich 2005, Pastar et al. 2014). In the outermost part of the skin (*stratum corneum*), keratinocytes are fully differentiated and cornified. Henceforth they are called corneocytes (König & Liebich 2005, Pastar et al. 2014). Corneocytes form a cover that finally acts as a protective barrier against the environment and maintains the fluid balance in the epidermis (Pastar et al. 2014). Besides keratinocytes, Merkel cells (tactile cells), Langerhans cells (immune cells) and melanocytes (pigment cells) are located in the epidermis (Welsch & Deller 2010). The dermis is located between the epidermis and the subcutaneous tissue. It is tightly connected to the epidermis through a basement membrane and divided into two layers: the *stratum papillare* (anchored in the epidermis) and the subjacent *stratum reticulare*. The dermis consists of a connective tissue which is rich in collagen and elastic fibres that gives the skin its flexibility and strength (König and Liebich 2005, Welsch and Deller 2010). It further contains nerve endings (to sense pain, touch, pressure, and temperature), hair follicles, and different types of glands, lymphatic vessels and blood vessels. The blood vessels provide nourishment and waste removal for both dermal and epidermal cells and play an important role in the thermoregulation of homoeothermic organisms (Welsch & Deller 2010). The subcutaneous tissue (*subcutis*) or hypodermis is located underneath the cutis and is not part of the skin. It mainly comprises of fatty tissue that serves as an energy reservoir, provides protective padding, and insulates the body from heat and cold (Welsch & Deller 2010).

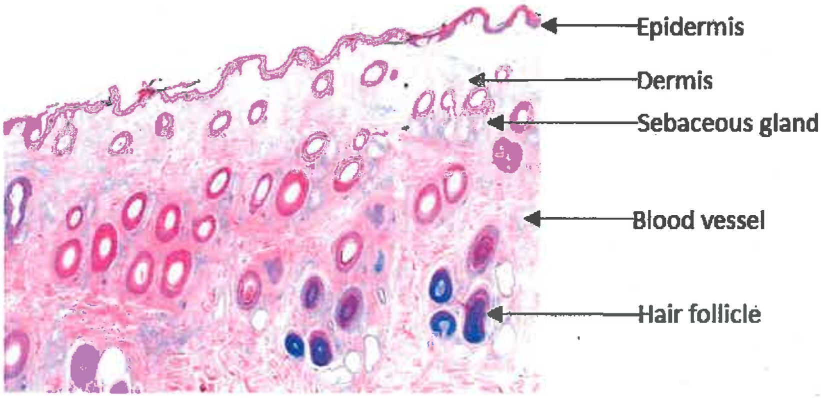


Figure 1: The cutis of a cow with its structural components (haematoxylin and eosin stained).

3.2 The skin and its key role in prevention of an inflammation

Once the skin is injured, the complex of wound healing is initiated (Gurtner et al. 2008, Rosique et al. 2015). This complex comprises of three stages: inflammation, new tissue formation and tissue remodelling (Gurtner et al. 2008). Inflammation appears directly after (skin) injury and is characterized by five classic signs: redness (*rubor*), swelling (*tumor*), heat (*calor*), pain (*dolor*) and loss of function (*functio laesa*) (Ryan & Majno 1977). Due to damage of the epidermis various signals are released from the wound site towards adjacent blood vessels. These react with vascular changes, such as vasodilation (widening of the blood vessels), increased permeability and increased blood flow (Ryan & Majno 1977). Vasodilation and the associated increase in blood flow cause the redness (*rubor*) and an increase in heat (*calor*). A rise in permeability of the blood vessels leads to an increased release of plasma into the tissues. This lastly results in swelling (*tumor*) (Ryan & Majno 1977). Furthermore, the endothelial cell junctions of the blood vessels become loose and allow leukocytes to transmigrate and move towards the wound. As soon as leukocytes have reached the wound, they get activated and release signals to attract more leukocytes (e.g. neutrophils, monocytes and macrophages), with the task to remove the breakdown products of the injured cells and clots (Martin & Leibovich 2005, Gurtner et al. 2008, Rosique et al. 2015). During this initial stage of wound healing, the organism needs all its components from the coagulation cascade, the inflammatory pathways and the immune system; in order to stop blood loss, to remove dead tissue and to prevent an infection (Gurtner et al. 2008). The second stage of wound repair (new tissue formation) starts two to ten days after injury (Gurtner et al. 2008). First, keratinocytes migrate into the wound and start to close the defect in the dermis (Pastar et al. 2014). Later, fibroblasts are attracted from the edge of the wound and start to produce collagen and extracellular matrix; the two main components of an animal tissue (Gurtner et al. 2008). In addition to that, fibroblasts have the ability to differentiate into contractile myofibroblasts. These, over the time, close the wound (Gurtner et al. 2008, Rosique et al. 2015). The contraction of the wound edges and the aberrant deposition of collagen finally form a scar (Nunan et al. 2014). On the basis of scarring it is possible to deduce the intensity and duration of the inflammatory process during wound healing (Rosique et al. 2015). The third and final stage of wound repair is "tissue remodelling". It starts two to three weeks after injury and can last for more than a year (Gurtner et al. 2008). In this stage all processes, that have been activated after skin injury, decrease and eventually stop; leaving a mass that mainly consists of collagen (Gurtner et al. 2008). Despite remodelling of the tissue only 70 percent of the tensile strength of normal/uninjured skin is regained (Rosique et al. 2015).

3.3 The skin and an unresolved acute inflammation

In some cases, a persistent and unresolved acute inflammation is followed by chronic inflammation. This is characterized by simultaneous destruction and healing of the tissue and a dominating presence of macrophages (Nunan et al. 2014). Macrophages are powerful defensive cells that release toxins to fight against invading pathogens. These toxins are not only harmful to the invaders but also to the organism itself. Therefore, a chronic inflammation is almost always accompanied by tissue destruction (Nunan et al. 2014). The morphology of the epidermis of a chronic wound (e.g. pressure ulcer) thus differs markedly from the morphology of the epidermis of a healthy skin (Pastar et al. 2014). Pressure ulcers are serious health problems in humans (Berlowitz & Brienza 2007, Kottner et al. 2009, Nunan et al. 2014) as well as in farm animals e.g. breeding sows (Herskin et al. 2011) and dairy cows (Müller 2004).

A pressure ulcer is characterized by a hyperproliferative epidermis, a hyperkeratosis (a thickened *stratum corneum*) and a parakeratosis (presence of the nuclei in the cornified layer) (Stojadinovic et al. 2005, Pastar et al. 2014). According to the National Pressure Ulcer Advisory Panel (NPUAP 2016) a pressure ulcer is a localized damage to the skin and/or underlying soft tissue usually over a bony prominence, displayed as an intact skin or an open ulcer and a result of intense and/or prolonged pressure or pressure in combination with shear. According to Berlowitz & Brienza (2007) and Kottner et al. (2009), compressive and shear forces seem to primarily affect deeper tissue layers of which muscles showed the lowest tolerance to pressure (Linder-Ganz et al. 2006, Kottner et al. 2009). Prolonged local pressure may result in vascular occlusion and local ischemia (Bansal et al. 2005). That in turn, leads to an insufficient oxygen supply, a reduced availability of nutrients and an inadequate removal of metabolites in the affected tissue, which consequently results in an increased rate of cell death (necrosis) (Bansal et al. 2005). Also friction is considered as a risk factor for pressure injuries. It seems that friction cause rather superficial lesions than damages in the deep tissue layers. Anyhow, friction may contribute to/or exacerbate a pressure injury development due to the shear forces that it creates (Berlowitz & Brienza 2007, Kottner et al. 2009, Brienza et al. 2015). In summary, externally applied pressure will rather increase damages in the deep tissues near bony prominences than cause damages in the superficial tissues close to the skin's surface (Berlowitz & Brienza 2007).

3.4 Thermoregulation and heat dissipation in warm blooded organisms

Homoeothermic organisms have the ability to maintain a constant (internal) body temperature in response to various internal and external thermal stimuli through heat generation (shivering) and heat dissipation (cutaneous vasodilation and sweating) (Charkoudian 2003, Silbernagl & Despopoulos 2012). In warm-blooded organisms heat can be emitted through four different mechanisms, namely: conduction, convection, radiation and evaporation (Kadzere & Murphy 2002, Silbernagl & Despopoulos 2012). Heat emission through the skin depends on the temperature gradient between the organism and its environment and shifts from radiation and convection at lower environmental temperatures to vaporization at higher temperatures (Kadzere & Murphy 2002). However, the main proportion of the heat is emitted through the skin by radiation (Silbernagl & Despopoulos 2012). Any object with temperature above zero emits infrared radiation with a wavelength between 1 mm and 780 nm (Lahiri et al. 2012). Infrared radiation can be perceived as heat and is therefore also called thermal radiation (Eddy & Snyder 2001, Lahiri et al. 2012, Redaelli et al. 2014). The emitted heat (thermal radiation) of a homoeothermic organism directly reflects the underlying circulation and the tissue metabolism rate. Thus, areas with a high metabolism rate and major blood vessels appear warmer than areas with less tissue activity or regions distant from major blood supply (Eddy & Snyder 2001, Nikkhah et al. 2005). Heat is one of the five signs of an inflammation (see above) and is typically characterised by an increase in the permeability of blood vessels and an concurrent increase in blood flow (Ryan & Majno 1977). This finally leads to an alteration of the thermal pattern of the skin (Alsaaod et al. 2015).

3.5 Thermography as a diagnostic tool to detect thermal abnormalities

Temperature is a proven scientific indicator of health and the correlation between temperature and disease is very well studied (Silbernagl & Despopoulos 2012). Therefore, infrared thermography is used in human medicine as well as in veterinary medicine to detect, to localize and to visualize superficial thermal changes caused by inflammations and/or injuries (Eddy et al. 2001, Lahiri et al. 2012, Alsaaod et al. 2015). In the case of infrared thermography the emitted thermal radiation (heat) of an object can be measured by means of an infrared camera. This device converts infrared radiation into temperature values and displays the information as an image, named thermogram (Lahiri et al. 2012, Alsaaod et al. 2015). A thermogram is normally displayed in a colour scale. Each pixel within the thermogram reflects the measured surface temperature or intensity of the emitted radiation of an object. Usually, the hottest areas are represented in bright colours, while the cooler areas appear in dark colours (Alsaaod et al. 2015). The major advantage of infrared thermography is, that it is non-invasive. Furthermore it is non-contacting, fast and passive (Lahiri et al. 2012). It has no harmful radiation effects and is extremely sensitive (Lahiri et al. 2012, Alsaaod et al. 2015); variations of ± 0.1°C can be detected (Alsaaod et al. 2015). On one hand, this metrological characteristic makes it possible to detect changes in skin temperature at a very early stage, on the other hand, thermographic measurements are easily influenced by ambient temperature, air flow, sunlight, moisture and individual animal variations (Lahiri et al. 2012). Therefore, whenever infrared thermography is used as a diagnostic tool it is of major importance to measure under controlled environmental conditions (Lahiri et al. 2012, Alsaaod et al. 2015). In veterinary medicine, infrared thermography has been used to detect lameness and inflammation in horses (Eddy & Snyder 2001) and in cattle (Alsaaod et al. 2015). It was further applied to examine cattle infected with foot and mouth disease (Rainwater-Lovett et al. 2009). Berry et al. (2003) used it as a diagnostic technique to detect mastitis in dairy cows. In most of these cases, infrared thermography made it possible to detect thermal variations before clinical signs were present and to intervene at a very early stage. Although infrared thermography has been successfully applied it has been recommended to use it in combination with other methods rather than as a replacement for them (Lahiri et al. 2012, Alsaaod et al. 2015).

3.6 Integumentary alterations as animal-based parameters of welfare

The environment of dairy cow has a substantial effect on its health and welfare (Green et al. 2012). Therefore, on farm welfare monitoring and surveillance systems for dairy cows have a strong emphasis on animal-based measures, such as behaviour, health and physiology (Whay et al. 2003, Efsa 2012). Animal-based parameters reflect an animal's reactions to their specific environment more directly than the physical features of the housing (environment-based parameters) (Whay et al. 2003). Particularly, measures of body damages are relevant to welfare assessment (Broom 1991), and allow to compare the welfare status of both the herd and the individual (Gibbons et al. 2012). Once an injury occurs, the state of the animal is affected and the welfare is poor. The welfare would be even poorer when the injury is accompanied by pain (Broom 1991). According to Broom (1991) an injury itself is an indicator of poor welfare. Thus, integumentary alterations reflect a decreased welfare of a dairy cow (Brenninkmeyer et al. 2016).

3.6.1 Hock lesions common skin alterations in housed dairy cows

Hock lesions are the most common skin alterations in housed dairy cows (Weary & Taszkun 2000, Keil et al. 2006, Fulwider et al. 2007, Rutherford et al. 2008, Kielland et al. 2009, Nuss & Weidmann 2013, Brenninkmeyer et al. 2016). They mostly occur on the lateral aspects of the tarsal joint (Weary & Taszkun 2000, Lim et al. 2015) and it is assumed that they are caused by repeated conflicts of the animal with its environment (Kester et al. 2014, Brenninkmeyer et al. 2016). The term hock lesion stands for various clinical presentations of hock injuries, including the three major appearances hair loss, ulceration and swelling (Regula et al. 2004, Rutherford et al. 2008, Kielland et al. 2009, Laven & Livesey 2011, Potterton et al. 2011a, Brenninkmeyer et al. 2013, Burow et al. 2013, Lim et al. 2015). Among these alterations, hair loss is the most prevalent one (Huxley et al. 2004, Potterton et al. 2011a). While previous studies have primarily focused on risk factors, there is still a lack of understanding regarding the aetiology and development of hock lesions (Kester et al. 2014, Lim et al. 2015). Furthermore, the precise impact of hock lesions on the dairy cow´s welfare is largely unknown (Laven & Livesey 2011). Nevertheless, it is reasonable to assume that the severity/extent of hock injuries reflects the degree of discomfort associated with the housing system (Rutherford et al. 2008, Kester et al. 2014, Brenninkmeyer et al. 2016). Livesey et al. (2002) reported that hair loss alone might be no welfare problem but displays (mild) abrasion, that is caused by high local pressure or friction on hard and abrasive lying surfaces (Nuss & Weidmann 2013). However, it may become welfare relevant as soon as the skin is injured (haemorrhage and/or a scab formation) (Livesey et al. 2002, Kester et al. 2014). Firstly, cutaneous lesions are considered to be painful (Vinuela-Fernandez et al. 2007) and secondly skin injuries are a possible entrance for pathogens (Müller 2004, Kester et al. 2014, Clegg et al. 2016). Pathogenic organisms may cause an inflammation and especially under unhygienic circumstances there is a high risk that an infection shifts from non-bacterial to bacterial (Herskin et al. 2011, Kester et al. 2014). Bacteria may spread via circulatory or lymphatic systems and may cause severe health problems such as pyaemia (a type of sepsis) or bacterial endocarditis or even death (Kester et al. 2014, Clegg et al. 2016). Hock lesions can also lead to an infection of the underlying tissues especially of synovial structures such as tendon sheaths or bursae. That may result in a tendovaginitis or a hygroma formation (swelling) (Müller 2004, Kester et al. 2014). Both are considered to be very painful (Müller 2004, Kester et al. 2014). Furthermore, in cases where lesions become infected, purulent and are accompanied by a severe swelling of the joint lameness was increasingly observed (Brenninkmeyer et al. 2013, Kester et al. 2014).

3.6.2 Hock lesions and issues with the scoring systems

Hock injuries are common in cubicle housed dairy cows. Thus, several studies focused on the prevalence of hock lesions (Kester et al. 2014). Weary & Taszkun (2000) and Lombard et al. (2010) determined a prevalence of 73% and 24% in Canada and in the United States, respectively. In European assessments, a prevalence of 8% in Switzerland (Regula et al. 2004), 46% in the United Kingdom (Rutherford et al. 2008), about 50% in Denmark (Klaas et al. 2003), 61% in Norway (Kielland et al. 2009) and approximately 65% in Austria and Germany (Brenninkmeyer et al. 2013) have been reported. However, these studies may only be compared to a very limited extent (Laven & Livesey 2011) due to different scoring systems used. Either all cases or only the most severe presentation of hock lesions are reported (Laven & Livesey 2011, Kester et al. 2014). Some studies (Potterton et al. 2011a, Brenninkmeyer et al. 2016) provide data on the specific prevalence for each clinical presentation of hock lesions: hair loss, ulceration and swelling (Kester et al. 2014). In the study of Potterton et al. (2011), an extended version of a scoring system was used, called "hock map". According to a four-point scale they investigated both tarsal joints (left and right) at two locations (hock and the tuber calcis) for hock lesions and reported a total prevalence for hair loss, ulceration and swellings of 82%, 17% and 100%, respectively. Another example, where the specific prevalence for each clinical presentation of hock lesions was reported is a study conducted by Brenninkmeyer et al. (2016). In this investigation all alteration that were detectable at the tarsal joint (including the hock and the tuber calcis) were noted regardless of the size; a total prevalence for hair loss, ulceration and swellings of 68%, 48% and 40%, respectively was found.

Despite the limitations with regard to the comparability of scoring systems and the various definitions of hock lesions, it could be clearly shown that hock lesions are highly prevalent in cubicle housed dairy cows (Kester et al. 2014). However, for the future it is important that injury scoring scales are standardised, validated and simple (Laven & Livesey 2011, Gibbons et al. 2012).

3.6.3 Hock lesions and their (possible) risk factors

Several studies focused on the identification of risk factors associated with hock lesions in dairy cows (Regula et al. 2004, Keil et al. 2006, Rutherford et al. 2008, Kielland et al. 2009, Potterton et al. 2011, Brenninkmeyer et al. 2013). Such factors are commonly divided into two main groups: cow-related risk factors and housing-related risk factors (Kester et al. 2014). As cow-related risk factors, lameness, age, body condition score, cow size, hygiene, milk yield, stage of lactation and breed have been identified (Kester et al. 2014). The housing system, cubicle dimensions and other properties of the cubicles have been assigned to housing-related risk factors (Kester et al. 2014). Despite these studies the actual relationship between housing design, cow- and also management related risk factors appears to be complex and interrelated (Kester et al. 2014). However, bedding material (Potterton et al. 2011a) and lameness (Kielland et al. 2009, Potterton et al. 2011, Brenninkmeyer et al. 2013) may have a key role in the development of hock lesions. A causal relationship, where the presence of hock lesions leads to an increased risk of lameness, has so far not been demonstrated (Kester et al. 2014). Nevertheless, improvements in the dairy cows' lying comfort may contribute to a decline of the prevalence for hock lesions. This in turn may lead to a reduction in lameness and may further be beneficial for the dairy cows' welfare (Kester et al. 2014, Brenninkmeyer et al. 2016).

4 Animals, material and methods

The project consisted of two separate parts to investigate hock lesions in lactating dairy cows. In both studies, in agreement with other studies (e.g. Rutherford et al. 2008, Potterton et al. 2011a) the term "hock lesions" is used which comprises both hair loss (with different degrees of severity/extent) and ulceration (characterised by areas of broken skin or scabs).

4.1 Study 1

In July 2015, the first study was carried out in cooperation with the University of Life Science Warsaw on a free-stall dairy farm in Poland. The farm kept about 350 lactating Holstein dairy cows out of which 172 were investigated regarding hock lesions. Each dairy cow was examined individually with regard to hair loss and ulceration at the lateral hock and at the lateral point of the hock (tuber calcanei). The examination comprised visual scoring and thermal imaging and was performed in the milking parlour (herring bone type) during the afternoon milking sessions. Whether the right or left tarsal joint of a dairy cow was investigated, was determined by the cow entering the milking parlour (right or left side of the milking system). The degree of hair loss was scored according to a four-point categorical scale, which was modified after Potterton et al. (2011a) (Fig. 1). The criteria for the hair loss scoring were: hair undisturbed, no hair loss (0/normal); hair disturbed "curly", no hair loss (1/mild); hair loss ≤ 2.5 cm in diameter (2/moderate); hair loss ≥ 2.5 cm in diameter (3/severe). For easier distinction between the different degrees of severity of hair loss, we attached a sticker with a diameter of 2.5 cm as a reference point in the middle between the hock and the point of the hock. Ulceration was additionally recorded as absent (0) or present (1) (Fig. 2). The degree of severity of hair loss is hereinafter denoted as "HL" score.

HL Score	0	1	2	3
Definition	Normal	Mild	Moderate	Severe
Description	Hair undisturbed, no hair loss	Hair disturbed ("curly"), no hair loss	Hair loss ≤ 2.5 cm in diameter	Hair loss ≥ 2.5 cm in diameter
Example				

Figure 2: Specification of the different HL scores at the hock and point of the hock.

Score for ulceration	1	
Definition	Present	
Description	Broken skin	Scab
Example		

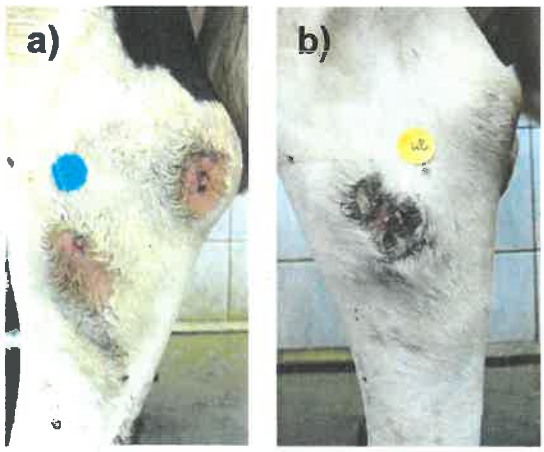


Figure 3: Specification of an ulceration at the hock and point of the hock; with an example of "broken skin" at the hock and the point of the hock (a) and an example of a "scab" at the hock (b).

Per dairy cow, four consecutive thermal images of the hock and the point of the hock were taken using an infrared camera (FLIR T440). The emissivity value (Ɛ) was set to 0.98 and all images were recorded at the same distance of approximately 0.7 m from the animal. The camera was held so that the hock or the point of the hock appeared clearly in the centre of the image. 10 hocks and 7 points of the hock not affected by hock lesions were dry-shaved with an electric shaver and used as control animals. ThermaCam Researcher Pro 2.10, an image analysing software, was used to determine temperature values from the thermal images. On each thermal image a region of interest was defined by drawing a circle around the affected area (Fig. 3). The region of interest consisted of 5145 and 1313 pixels at the hocks and at the points of the hock, respectively; each pixel reflected one temperature value (°C). In order to obtain information on the skin temperature in the region of interest, we decided to average the maximum temperature of the four images per animal and used that averaged value for further statistical analysis.

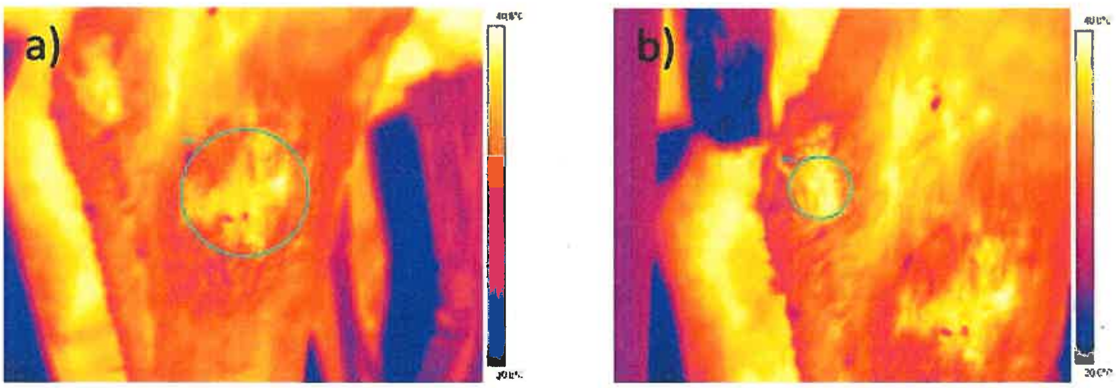


Figure 4: Thermal image of a hock (a) and a point of the hock (b) from the right side. Region of interest surrounded by a blue circle. Variations in skin temperature are represented by different colours: the hottest areas are white and the coolest areas black.

Each tarsal joint was additionally photographed using a digital camera (Canon PowerShot Pro1) and images were stored as JPEG (Joint Photographic Experts Group) files. For hocks and points of the hock with a HL score of 2 and 3 the area of hair loss in square centimetre (cm^2) was calculated with the image processing program ImageJ 1.49. In order to obtain measures in square centimetres, ImageJ needed the information what a pixel on the digital photograph represented in real size. Therefore, the width of the reference point with a known size of 2.5cm was measured in pixels using the straight line selection tool. Based on that information the programme computed a scaling factor, which allowed to convert an area measured in pixels into an area expressed as square centimetres. For each digital photograph an individual scaling factor was calculated. The freehand selection tool was used to mark the area of hair loss on the digital photograph by drawing an outline around it and the measure of that area in square centimetres was automatically displayed by the programme.

4.2 Study 2

The second study comprised visual scoring of hock lesions and histological examination. Sampling was carried out on an abattoir in Upper Austria in October 2015. At the abattoir, 25 hocks and 26 points of the hock of dairy cows were scored visually before slaughtering of the cows. Due to the design of the unloading facilities at the abattoir, only the left tarsal joint of the animals was available for scoring. Visual scoring was conducted as described in Study 1. After slaughtering and skinning, skin samples of about 3 x 3 cm were taken from the animals that had been scored before slaughter (identification by ear tag number). Samples were put into a container with 10% neutral buffered formalin for fixation and were brought to the lab. Histological preparation and investigation of the samples was carried out at the Institute of Pathology and Forensic at the University of Veterinary Medicine in Vienna during November 2015 and January 2016. In brief, skin samples were embedded in paraffin, cut with a microtome into 5µm sections and stained with haematoxylin and eosin. The histological specimens were inspected under a light microscope at 2 times magnification (Olympus BX50), photographed with a digital microscope camera (Leica EC3) and scored according to a four-point categorical scale for the degree of tissue alteration. The scale was modified after Michel et al. (2012) (Fig. 4) and the criteria for the tissue alteration scoring were: no lesion, no hyperplasia and/or hyperkeratosis of the epidermis (0/normal); mild to moderate hyperplasia and/or hyperkeratosis of the epidermis, mild inflammatory infiltration (1/mild); marked hyperplasia and hyperkeratosis of the epidermis followed by fibrosis, marked inflammatory infiltration (2/moderate); full-thickness necrosis of the epidermis and/or purulent inflammation (3/severe). The degree of severity of tissue alteration is hereinafter denoted as "TA" score.

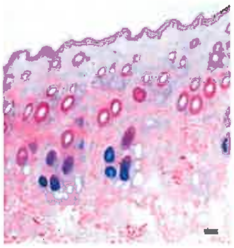
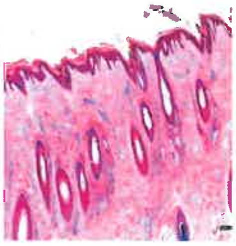
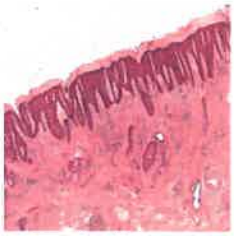
TA Score	0	1	2	3
Definition	**Normal**	**Mild**	**Moderate**	**Severe**
Description	No lesion, no hyperplasia and/or hyperkeratosis of the epidermis	Mild to moderate hyperplasia and/or hyperkeratosis of the epidermis, mild inflammatory infiltration	Marked hyperplasia and hyperkeratosis of the epidermis followed by fibrosis, marked inflammatory infiltration	Full-thickness necrosis of the epidermis and/or purulent inflammation
Example				

Figure 5: Specification of the different TA scores at the hock and point of the hock.

4.3 Statistical analysis

The effects of hair loss on the skin temperature of both, the hock and point of the hock were tested by means of Kruskal-Wallis test and Nemenyi's post-hoc test. An influence of ulcerations on skin temperature within a score class was further tested by means of Wilcoxon rank sum test. The association between skin temperature and the extent of hair loss was tested by linear regression. Fisher´s exact test was performed to test if histological scores are reflected in visual scores and vice versa. All statistical analyses were performed in R (R Core Team 2015). The level of significance was set at a p-value < 0.05.

5 Results

5.1 Study 1

5.1.1 *Prevalence of skin alterations*

In total 172 tarsal joints were examined for hock lesions. A HL score of 0, 1, 2 and 3 was allocated to 2%, 20%, 19% and 59% of the hocks, respectively. Hocks with a HL score of 2 and 3 had on average an area of hair loss of 4.0 cm² (SD 2.4) and 14.1 cm² (SD 8.0), respectively. Twelve percent of the animals with a HL score of 2 and 23% with a HL score of 3 also showed ulceration (Fig. 5 a). Twenty six percent, 30%, 26% and 19% of the points of the hock received a HL score of 0, 1, 2, and 3, respectively. At the points of the hock, the area of hair loss was on average 3.8 cm² (SD 2.2) and 9.1 cm² (SD 3.9) for HL scores 2 and 3, respectively. At the points of the hock, 9% of the dairy cows with a HL score of 2 and 18% with a HL score 3 also exhibited signs of ulceration (Fig. 5 b).

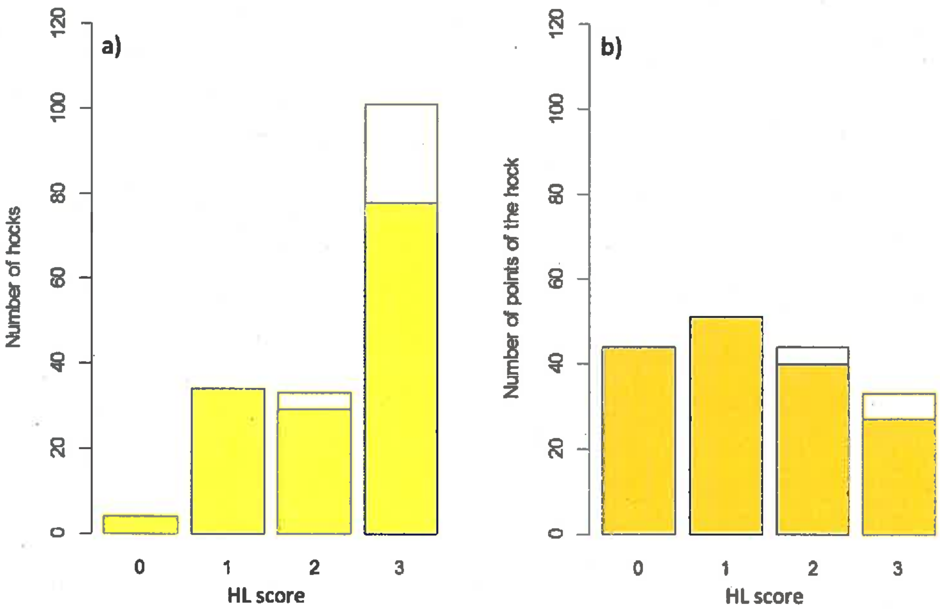


Figure 6: Total number of hocks (a) either without ulceration (yellow) or combined with ulceration (grey) and total number of points of the hock (b) either without ulceration (orange) or combined with ulceration (grey) within the different scores for HL.

5.1.2 *Skin temperature*

The average maximum skin temperature of control animals was 36.5°C (SE 0.2) at the (shaved) hocks. For hocks with a HL score of 2 and 3 the average maximum skin temperature was 37.7°C (SE 0.1) and 37.8°C (SE 0.1), respectively (Fig. 6 a). Hocks and points of the hock with a HL score of 0 and 1 were not further considered in statistical analysis, due to the insulating effect of the hairs. The maximum skin temperature of animals with a HL score of 2 and 3 was significantly higher compared to control animals. However, there was no significant difference in the skin temperature between hocks with a HL score of 2 and 3. At the points of the hock, the average maximum skin temperature was 36.2°C (SE 0.3) for control animals and, 37.3°C (SE 0.1) and 37.4°C (SE 0.1) for animals with a HL score of 2 and 3, respectively (Fig. 6 b). The maximum skin temperature was significantly higher at points of the hock with a HL score of 2 and 3 compared to control animals. Again, there were no significant differences in the maximum skin temperature between points of the hock with a HL score of 2 and 3.

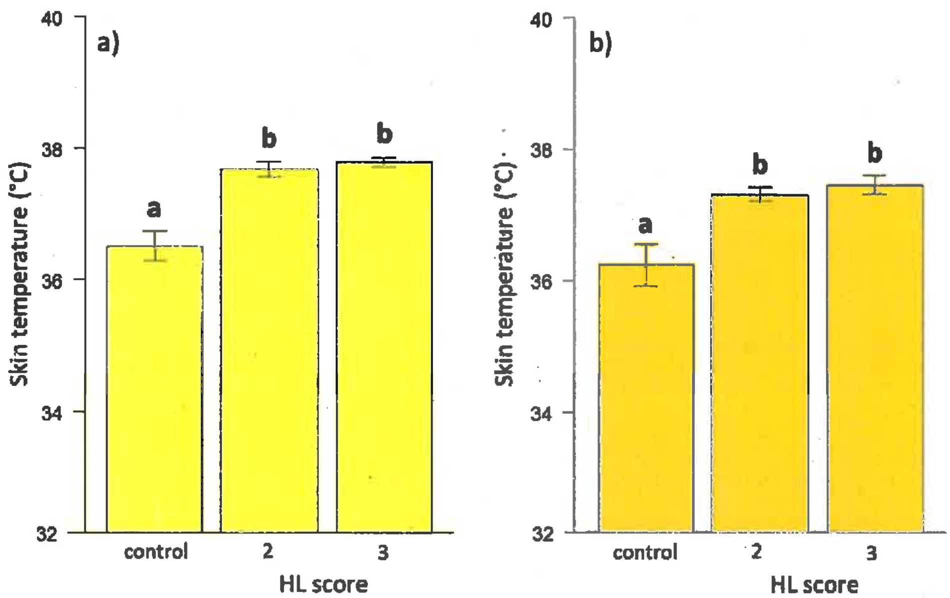


Figure 7: Maximum skin temperature at the hocks (a) and the points of the hock (b) of control animals and of animals with HL scores of 2 and 3. The error bars represent the standard error of the mean.

There was no significant difference in the maximum skin temperature between hocks without (37.7°C (SE 0.1), n=29) and with (37.6°C (SE 0.4), n=4) ulceration with a HL score of 2 (Fig. 7 a). However, within HL score 3 the skin temperature at hocks with ulceration (38.1°C (SE 0.1), n=23) was significantly higher than in hocks without ulceration (37.7°C (SE 0.1), n=78; Fig. 7 b).

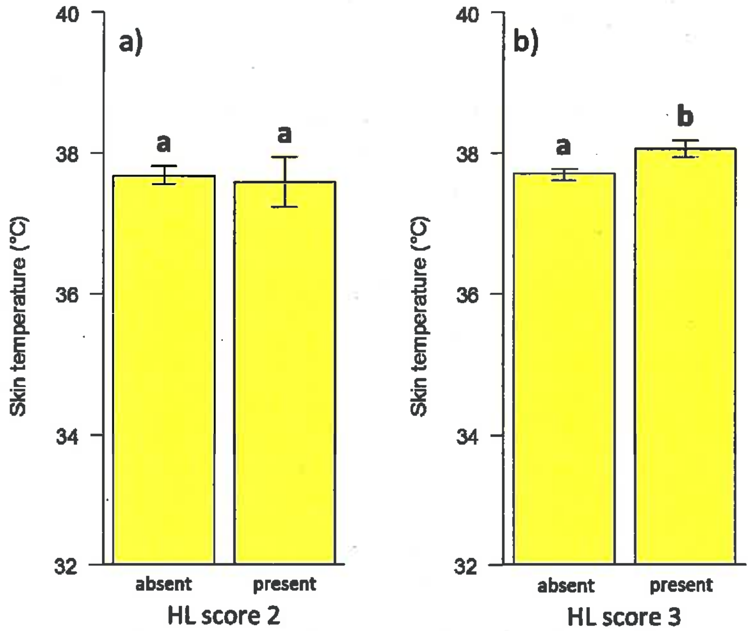


Figure 8: Maximum skin temperature at the hocks without (absent) and with ulceration (present) within the HL scores 2 (a) and 3 (b). The error bars represent the standard error of the mean.

There was no significant difference in maximum skin temperature between points of the hock without (37.3°C (SE 0.1), n=40) and with (37.1°C (SE 0.3), n=4) ulceration within HL score 2 (Fig. 8 a). Additionally, there was no significant difference in the maximum skin temperature between points of the hock without (37.3°C (SE 0.2), n=27) and with (38.0°C (SE 0.2), n=6) ulceration within HL score 3 (Fig. 8 b).

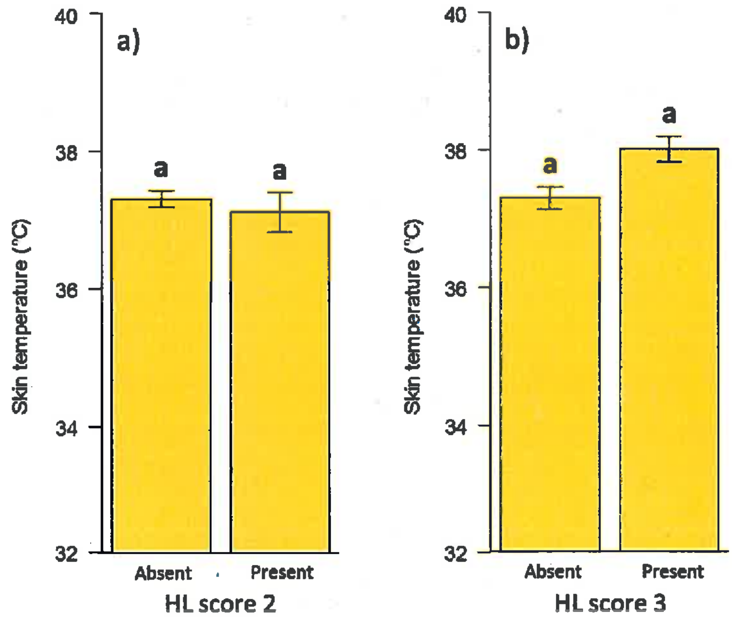


Figure 9: Maximum skin temperature at the points of the hock without (absent) and with ulceration (present) within the HL scores 2 (a) and 3 (b). The error bars represent the standard error of the mean.

5.1.3 Proportion of pixels above the reference threshold value (HL score 2 and 3 including ulceration)

In 45% of the hocks, at most 10% of the pixels showed a temperature higher than 36.5°C, the latter representing the average maximum skin temperature of control cows at the hock and therefore chosen as reference threshold (Fig. 9 a). In 65%, 82%, 90%, 96%, 98% of the hocks, at most 20%, 30%, 40%, 50%, and 60% of the pixels showed a temperature higher than 36.5°C. Twenty seven percent of the points of the hock had at most 10 % of the pixels above the reference threshold (36.2°C, average of the maximum skin temperature of control cows at the point of the hock) (Fig. 9 b). For 41%, 54%, 64%, 77%, 87%, 91%, 94% and 97 % of the points of the hock, at most 20%, 30 %, 40 %, 50%, 60%, 70%, 80% and 90% of the pixels showed a temperature higher than 36.2°C.

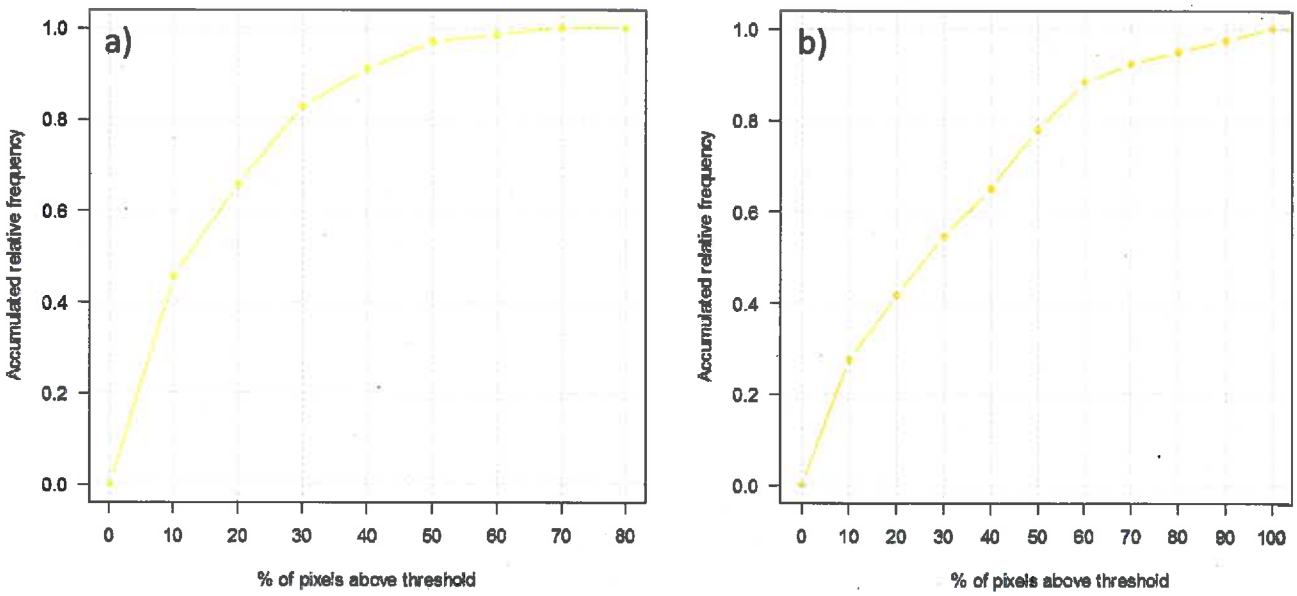


Figure 10: Accumulated relative frequency of hocks (a) and of points of the hock (b) in terms of the percentage of pixels above the reference threshold value derived from sound animals. The threshold for the hocks is 36.5°C and the threshold for the points of the hock is 36.2°C; only animals with HL score 2 and 3 were considered.

5.1.4 Associations of the amount of pixels above the reference threshold with the extent of hair loss (HL score 2 and 3 including ulceration)

There was a significant positive correlation between the number of pixels above the reference threshold and the extent of hair loss (cm²) for both hocks and points of the hock with a R^2 of 18% for hocks and a R^2 of 27% for points of the hocks (Fig. 10 a, b).

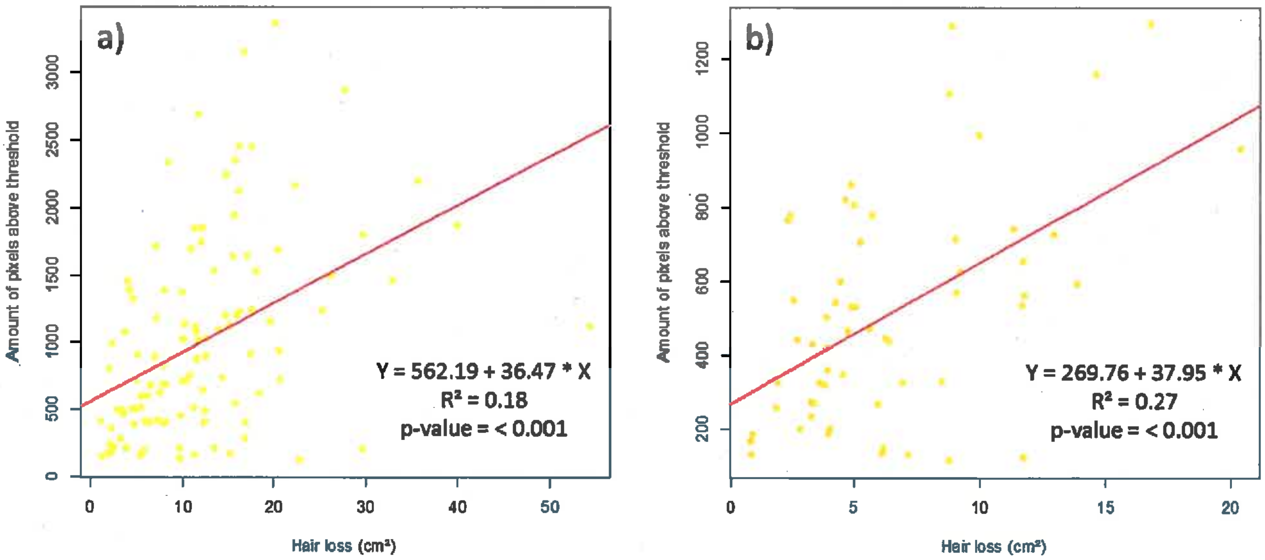


Figure 11: Relationship between the extent of hair loss (cm²) and the number of pixels above the reference threshold for the hocks (a) and points of hock (b) for the HL scores 2 and 3 including ulcerations. The threshold for the hocks is 36.5°C and for the points of the hocks 36.2°C.

5.1.5 Associations of the average temperature of pixels above the reference threshold with the number of pixels above the reference threshold (HL score 2 and 3)

When considering hocks without ulcerations only, the number of pixels above the reference threshold and the average temperature of pixels above the reference threshold was positively associated (R^2=0.45). This association was stronger when hocks with ulcerations were taken into account with a (R^2=0.74) (Fig. 11 a, b).

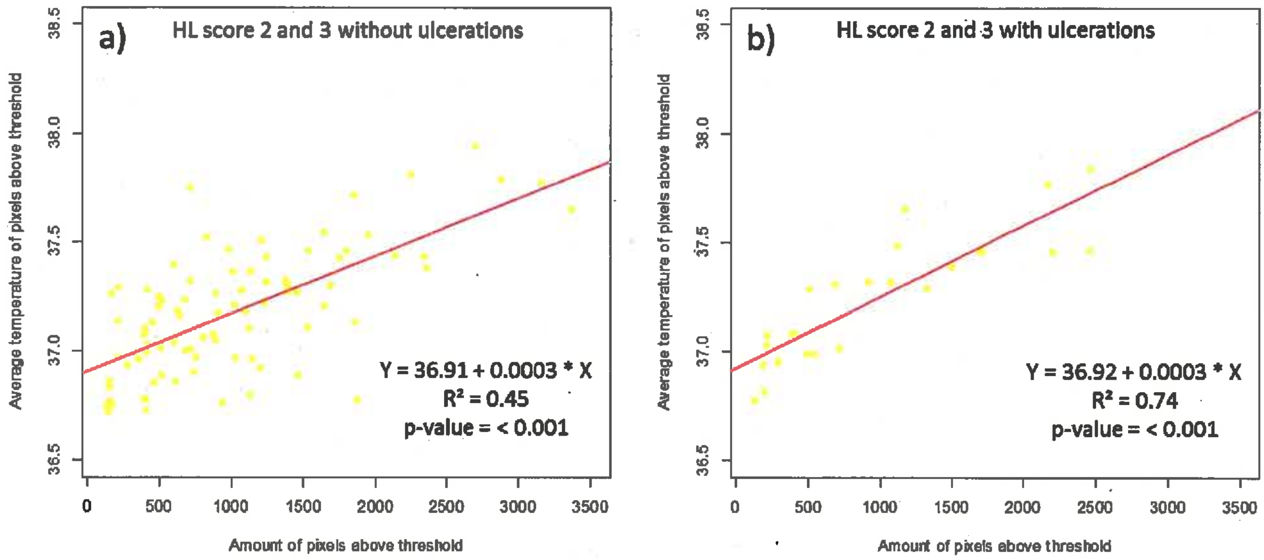


Figure 12: Relationship between the number of pixels above the reference threshold and the average temperature of pixels above the reference threshold for the hocks with a HL score of 2 and 3 without ulcerations (a) and the hocks with a HL score of 2 and 3 with ulceration (b). The reference threshold is 36.5°C for the hock with and without ulceration.

When considering points of the hock without ulcerations only, the number of pixels above the reference threshold and the average temperature of pixels above the reference threshold was positively associated (R^2=0.40). This association was stronger when points of the hock with ulcerations were taken into account with R^2=0.92 (Fig. 12 a, b).

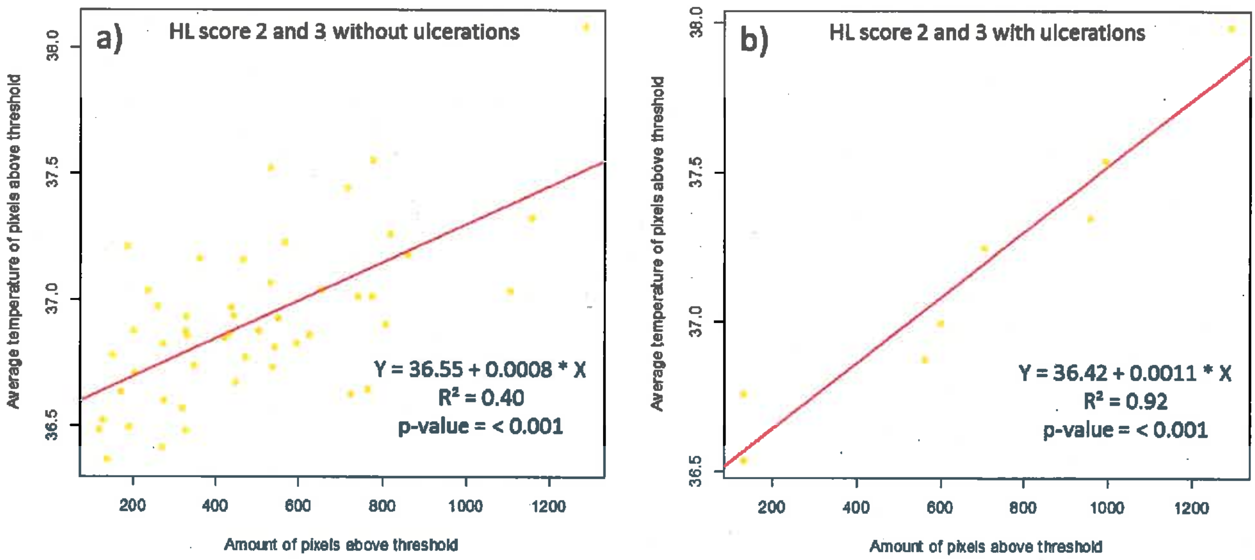


Figure 13: Relationship between the number of pixels above the reference threshold and the average temperature of pixels above the reference threshold for the points of the hock with a HL score of 2 and 3 without ulcerations (a) and the points of the hock with a HL score of 2 and 3 with ulceration (b). The reference threshold is 36.2°C for the points of the hock with and without ulceration.

5.2 Study 2

5.2.1 Prevalence of HL scores (visual examination)

Eight percent, 48%, 12% and 32% of the hocks received a HL score of 0, 1, 2, and 3, respectively. Thirty three percent of the animals with a HL score of 2 and 13% with a HL score of 3 also showed ulceration (Tab. 1). Nineteen percent, 35%, 15% and 31% of the points of the hock were assigned to a HL score of 0, 1, 2, and 3 respectively. Thirty eight percent of the points of the hock with a HL score of 3 also showed ulceration (Tab. 2).

Table 1: Number of hocks (n=25) allocated to the different HL scores; "+ ulc" represents the number of hocks that also showed ulcerations.

HL score	0	1	2	3
n	2	12	3	8
+ ulc	0	0	1	1

Table 2: Number of points of the hock (n=26) allocated to the different HL scores; "+ ulc" represents the number of points of the hock that also showed ulcerations.

HL score	0	1	2	3
n	5	9	4	8
+ ulc	0	0	0	3

5.2.2 Prevalence of TA scores (histological examination)

Sixteen percent, 40%, 40% and 4% of the hocks were assigned to a TA score of 0, 1, 2, and 3, respectively (Tab. 3). Eight percent, 38%, 42% and 12% of the points of the hock were allocated to a TA score of 0, 1, 2, and 3 respectively (Tab. 4).

Table 3: Number of hocks (n=25) allocated to the different scores of TA.

TA score	0	1	2	3
n	4	10	10	1

Table 4: Number of points of the hock (n=26) allocated to the different scores of TA.

TA score	0	1	2	3
n	2	10	11	3

5.2.3 *Associations of the degree of severity of hair loss and the degree of severity of tissue alteration*

Table 5: Contingency table of the HL scores and TA scores of the hocks (n=25). The table displays the numbers of the different combinations of HL scores and TA scores.

HL score / TA score	0 Normal Hair undisturbed, no hair loss	1 Mild Hair disturbed, no hair loss	2 Moderate Hair loss ≤ 2.5 cm	3 Severe Hair loss ≤ 2.5 cm	Ulceration Present Broken skin, scab	Σ
0 Normal No alteration	2 (100%)	2 (17%)	0 (0%)	0 (0%)	0 (0%)	4 (16%)
1 Mild Mild - moderate hyperplasia and/or hyperkeratosis, mild inflammation	0 (0%)	8 (67%)	1 (50%)	1 (14%)	0 (0%)	10 (40%)
2 Moderate Marked hyperplasia and hyperkeratosis, fibrosis, inflammation	0 (0%)	2 (17%)	1 (50%)	6 (86%)	1 (50%)	10 (40%)
3 Severe Necrosis of the epidermis and/or purulent inflammation	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (50%)	1 (4%)
Σ	2 (100%)	12 (100%)	2 (100%)	7 (100%)	2 (100%)	25 (100%)

A total number of 25 hocks were individually scored for hair loss and tissue alteration. 100% of the hocks that were visually scored with 0 (no hair loss) were also histologically scored with 0 (no alteration). 84% of the hocks that were visually scored with 1 (hair disturbed) were histologically scored with 1 or 2 (mild or moderate tissue alteration). 100% of the hocks with moderate hair loss and 100% of the hocks with severe hair loss were histologically scored with 1 or 2, respectively; the degree of tissue alteration increased with the size of the lesion. In 50% of the hocks, visual assignment of an ulceration was confirmed by histological examination. The association between the degree of severity of hair loss (columns) and the degree of severity of tissue alteration (rows) at the hocks is considered to be statistically significant (p=0.002, two-tailed Fisher's test).

Table 6: Contingency table of the HL scores and TA scores of the points of the hock (n=26). The table displays the numbers of the different combinations of HL scores and TA scores.

HL score / TA score	0 Normal Hair undisturbed, no hair loss	1 Mild Hair disturbed, no hair loss	2 Moderate Hair loss ≤2.5 cm	3 Severe Hair loss ≤2.5 cm	Ulceration Present Broken skin, scab	Σ
0 Normal No alteration	1 (20%)	1 (11%)	0 (0%)	0 (0%)	0 (0%)	2 (8%)
1 Mild Mild - moderate hyperplasia and/or hyperkeratosis, mild inflammation	4 (80%)	4 (44%)	1 (25%)	0 (0%)	1 (33%)	10 (38%)
2 Moderate Marked hyperplasia and hyperkeratosis, fibrosis, inflammation	0 (0%)	4 (44%)	3 (75%)	2 (40%)	2 (67%)	11 (42%)
3 Severe Necrosis of the epidermis and/or purulent inflammation	0 (0%)	0 (0%)	0 (0%)	3 (60%)	0 (0%)	3 (12%)
Σ	5 (100%)	9 (100%)	4 (100%)	5 (100%)	3 (100%)	26 (100%)

A total number of 26 points of the hock were individually scored for hair loss and tissue alteration. 20% of the points of the hock that were visually scored with 0 (no hair loss) were also histologically scored with 0 (no alteration). 88% of the points of the hock that were visually scored with 1 (hair disturbed) were histologically scored with 1 or 2 (mild or moderate tissue alteration). 100% of the points of the hock with moderate hair loss were histologically scored with 1 or 2, respectively. 60% of the points of the hock with severe hair loss were histologically scored with 3. The ulcerations that had been visually detected could not be confirmed by histological examination. The association between the degree of severity of hair loss (columns) and the degree of severity of tissue alteration (rows) at the points of the hock is considered to be statistically significant (p=0.031, two-tailed Fisher's test).

6 Discussion

6.1 Prevalence of skin alterations

In the present study, prevalence of hock lesions was high in the sample of dairy cows (Fig. 6a, b). While the majority of hocks were severely affected by hair loss, the amounts of affected points of the hock were almost equally distributed among all score classes. In total, 78% of the hocks and 50% of the points of the hock were hairless and 16 % and 6% also showed ulceration, respectively. In terms of order of magnitude, these findings are in agreement with other studies from Europe (Regula et al. 2004, Rutherford et al. 2008, Kielland et al. 2009, Rouha-Mülleder et al. 2010, Potterton et al. 2011, Brenninkmeyer et al. 2013) and North America (Weary & Taszkun 2000, Fulwider et al. 2007) that previously investigated hock lesions. However, it is difficult to compare prevalence values of hock lesions across studies; primarily due to a lack of standardized methodologies (see Laven & Livesey 2011).

6.2 Scoring scale

Gibbons et al. (2012) showed that simpler scoring scales can provide more reliable results than precise ones and further recommended the use of an already established scoring scale for the investigation of hock lesions. Thus, we used the "hock map" suggested by Potterton et al. (2011) for categorical scoring of hock lesions. In relation to the objective, we slightly adjusted the scoring-scale, i.e. we included the category "curly" hair (disturbed hair, but no hair loss), to particularly address mild hock lesions. Lim et al. (2015) reported, that an assessment of hock lesions using the hock map was rather time consuming. Therefore, we reduced the number of details included in the hock map in order to make scoring feasible during milking. Firstly, we only assessed the lateral side of one tarsal joint of each cow (because hock lesions most frequently occur on the lateral side of the tarsus (Weary & Taszkun 2000); secondly, we just differentiated (within the categories for hair loss) between hairless areas smaller or bigger than 2.5 cm in diameter using reference points; and thirdly, we recorded ulceration simply as present or absent. Moreover, we took a photograph of each hock and point of the hock instead of drawing them (Potterton et al. 2011a) and determined the exact size of the hairless area later on.

Through these modification scoring could be performed in the milking parlour without any temporal problems during milking.

6.3 Thermographic measures

Since we were not permitted to shave the hair of animals with a HL score of 1 at both hocks and points of the hock (='curly' hair), it was not possible to assess the thermal radiation of the skin in the affected areas due to the insulating effect of the hairs (Alsaaod et al. 2015). Therefore, this score class had to be excluded from statistical analysis with regard to thermographic measures. Nevertheless, we were allowed to shave 10 hocks and 7 points of the hock (not affected by hock lesions) that served as controls. Results from the thermographic study showed that hocks and points of the hock with a HL score of 2 and 3 had a significantly higher skin temperature in comparison to the controls (Fig. 7a, b). This may be interpreted as a sign of inflammatory processes in the underlying tissue, as heat is one of the five signs of an inflammation (Ryan & Majno 1977). No difference in skin temperature was found when comparing hocks and points of the hock with a HL score of 2 and 3 respectively (Fig. 7a, b). This suggests that due to the fact that it might be inflamed any hairless area, regardless of its size, may be considered relevant to the animal. A detailed investigation of ulcerations revealed that ulceration had no effect on skin temperature at the hocks and points of the hock within small alterations (HL score 2; Fig. 8a, 9a). There are two potential explanations for that. Firstly, a rise in temperature could not be detected due to scab formation (insulating effect) (Alsaaod et al. 2015). Secondly, dirt was mistakenly classified as ulceration. In comparison, hocks with and without ulceration within HL score 3 differed significantly in skin temperature (Fig. 8b). A similar trend was also found for the points of the hock within HL score 3 (($p = 0.06$; Fig. 9b). A higher skin temperature with ulceration may be explained by a more intense inflammatory reaction. A closer look at the photographs, of hocks and points of the hock with a HL score of 3 and ulceration, further revealed that the majority of those were swollen. Although swellings were not part of this study, we want to point out that they should not be neglected. Especially when associated with bacterial infections, they could lead to serious health problems (Müller 2004, Brenninkmeyer et al. 2013, Kester et al. 2014). Further research is urgently needed to better understand the development and cause of this condition. As mentioned above, an inflammation is characterised by an increase in the permeability of blood vessels and an concurrent increase in the blood flow (Ryan & Majno 1977), that finally leads to an alteration of the thermal pattern of the skin (Lahiri et al. 2012, Alsaaod et al. 2015).

In order to differentiate between normal (healthy) tissue and affected (inflamed) tissue, we set a reference threshold, which was deduced from the average maximum skin temperature of the control animals. The reference threshold was 36.5°C for the hocks and 36.2°C for the points of the hock. Any pixel that exceeded these thresholds was classified as inflamed. Based on that, we were able to quantify the number of pixels above the reference value, and were further able to calculate the proportion of 'inflamed' pixels within the defined region of interest. This gave us an idea about the size of the inflamed area within a hairless area at the tarsus. A small proportion of the hocks (7%) and points of the hocks (4%) had no pixels over the reference threshold (Fig. 10a, b). That means they were hairless, but did not show no inflammation according to our definition. There are two possible explanations for this finding: firstly, the hairs had simply been rubbed off without any change of thermal patterns of the skin. Or secondly, hair follicles have been displaced by fibrosis caused by a chronic inflammatory process, which is not necessarily followed by an increased heat dissipation (Berlowitz & Brienza 2007). However, in the animals with pixels above the threshold, we found a positive relation between the extent of hair loss and the amount of pixels above the reference threshold for both the hocks and the points of the hock (Fig. 11a, b). That means, that an increase in the area of hair loss is also positively associated with the size (in pixels) of the inflamed area. Furthermore, we could demonstrate a positive correlation between the number of pixels above the reference threshold and their average temperature at the hocks and the points of the hock with (12b, 13b) and without (12a, 13a) ulceration. On the basis of these results, we could affirm that an increase of the size (in pixel) of the inflamed area also leads to an increase in the superficial thermal patterns in that region. Such an effect became more evident at hocks and points of the hocks that showed an ulceration.

6.4 Tissue alteration

For histological scoring (see study 1) an already established scoring system was used, which was modified after Michel et al. (2012). Although originally developed for the assessment of footpad dermatitis in broiler chicken, the basic histopathological patterns of skin alterations may also be conferred to mammalian skin.

We investigated 25 hocks (Tab. 5) and 26 points of the hocks (Tab. 6) in terms of degree of severity of hair loss and degree of severity of tissue alteration. In agreement with Michel et al. (2012) we could show a significant correlation between visual und histological features of the skin. That means, in addition to a progressive loss of hair, the tissue increasingly altered with a potential development from a mild to a more severe state (Michel et al. 2012). Regarding the morphology of the epidermis (TA score 1 and TA score 2) the development of an epidermal hypertrophy (hyperkeratosis and hyperplasia) might have been caused by a repeated mechanical stress such as friction (Sanders et al. 1995). According to Mackenzie (1974) and Sanders et al. (1995), the degree of epidermal thickening can increase with an increased frictional stimulus. While higher levels of friction can result in ulceration, low levels of friction rather lead to adaptive thickening. Therefore, the epidermal hypertrophy within the samples might be understood as an adaptive reaction. Such a potential increase with time may be interpreted as a chronic response to recurrent irritation (Sanders et al. 1995). Through repeated rubbing and abrasion, the skin can rupture additionally (Sanders et al. 1995). Although low friction primarily causes superficial lesions, they should not be underestimated. On one hand such lesions can be painful (Müller 2004, Vinuela-Fernandez et al. 2007), and on the other hand they might cause a portal of entry for pathogens (Kester et al. 2014, Clegg et al. 2016). According to Wong et al. (2011) a persistent irritation of the skin may trigger a prolonged inflammation, which could lead to a non-physiological form of a fibrosis. Across samples with a TA score of 2, lymphocytes, plasma cells and fibroblasts indicated first signs of a chronic inflammation (Williams 1983). Furthermore, signs of pathological fibrosis (indicated by the formation of excess fibrous connective tissue), epithelial thickening and a lack of skin adnexa (Wynn 2007, Wong et al. 2011, Zeisberg & Kalluri 2013, Birbrair et al. 2014) were clearly evident. According to Wynn (2007) and Wong et al. (2011), alterations with a TA score of 2, might therefore be seen as a pathological state in response to mechanical stimulation. If the adaptation mechanisms of the skin fail, an abnormal mechanical loading may result in a breakdown of the surface tissue (Sanders et al. 1995). This can be seen at samples with a TA score of 3. Since lesions within this score class showed most of the histological features of chronic ulcers (e.g. full-thickness epidermal defect, fibrin deposition, neutrophilic infiltrate, necroinflammatory debris and a reactive thickening of the surrounding epidermis), these alterations might be interpreted as pressure ulcers (Witkowski & Parish 1982, Pastar et al. 2014). It may also be possible that skin lesions with a TA score of 3 have been caused by a traumatic injury followed by secondary bacterial infection (Ryan & Majno 1977). Tissue damages and the ensuing inflammatory response may promote hyperalgesia (Vinuela-Fernandez et al. 2007). Hyperalgesia can be related to changes in primary afferent neuron sensitivity which is induced by the production and release of chemical mediators from damaged cells. A pilot study in dairy cows revealed increased pain sensitivity with ulcerations at the tarsus but not in animals affected by hairless spots only (Winckler et al. 2014). However, further research regarding painfulness of hock lesions in dairy cows is needed.

7 Conclusion

This is the first study addressing the validity of hock lesions as welfare indicator in lactating dairy cows in terms of thermographic and histological aspects. We could show that already (an increase in) hair loss at a cow's hock and point of the hock results in (increased) hyperthermia and inflammatory infiltration. Based on the assumption that an inflammatory response is accompanied by pain, these findings support the hypothesis that already mild hock lesions are relevant for a dairy cow´s health and welfare. However, there are no studies available that especially focus on the actual consequences of hock lesions in terms of pain. Further research regarding painfulness, temporal development and aetiology of hock lesions in dairy cows is needed.

8 Summary

Hock lesions are common skin alterations in dairy cows. Previous studies have primarily focused on prevalence and respective risk factors. The precise impact of hock lesions on dairy cow's health and welfare, e.g. in terms of pain and suffering, is largely unknown. In order to investigate the relevance of hock lesions for dairy cow's welfare, we investigated signs of inflammation in different types of hock lesions, especially hyperthermia and the presence of inflammatory cells through thermal imaging and histological methods. We assumed that already mild hock lesions, indicated by hair loss, are relevant for an animal's welfare. The first study was carried out in cooperation with the University of Life Science Warsaw on a free-stall dairy farm in Poland. 172 tarsal joints of lactating dairy cows were investigated during milking. Two locations (hock and point of the hock) were visually scored on a four point categorical scale and thermal images (FLIR T440) were taken for skin temperature analysis. The second study comprised visual scoring and histological examination of 25 hocks and 26 points of the hock of dairy cows; sampling was carried out at an abattoir in Austria and histological evaluation was performed in cooperation with the University of Veterinary Medicine in Vienna. In the first study (visual and thermographic examination) an increase in hair loss (in terms of size of the area affected) resulted in an increase in skin temperature. This effect became more evident at hocks and points of the hocks that additionally showed ulceration. In the second study already mild hair loss indicated by disturbed/curly hair (no hair loss) was followed by mild tissue alterations. Moreover, an increase in hair loss was mostly reflected in an increased inflammatory response. These findings support our hypothesis that already mild hock lesions are followed by signs of an inflammation and are therefore relevant for dairy cow's health and welfare. However, further research regarding painfulness, temporal development and aetiology of hock lesions in dairy cows is needed.

9 Zusammenfassung

Schädigungen des Sprunggelenks wie Haarverluste, Ulzerationen und Schwellungen gehören zu den häufigsten Technopathien bei Milchkühen. Mit einer Prävalenz von größer als 50% stellen Sprunggelenkschäden im Hinblick auf das Wohlergehen der Milchkühe ein ernsthaftes Problem in der Laufstallhaltung dar. Auswirkungen dieser Schädigungen auf die Gesundheit und das Wohlergehen der Milchkühe, z.B. in Bezug auf Schmerzhaftigkeit und Leid, sind jedoch weitgehend unbekannt. Aufgrund dessen war das Ziel dieser Studie eine umfassende Validierung von Sprunggelenkschäden bei laktierenden Milchkühen. Dafür wurden haarlose Stellen und Ulzerationen seitlich am Tarsus und Fersenbeinhöcker in Bezug auf Entzündungsreaktionen mittels Infrarotkamera und histologischen Methoden untersucht. Die Arbeit wurde in zwei Studien unterteilt, wovon die erste Studie in Zusammenarbeit mit der naturwissenschaftlichen Universität Warschau (SGGW) auf einem Milchviehbetrieb in Polen durchgeführt wurde. 172 Sprunggelenke von laktierenden Milchkühen wurden während des Melkens untersucht. Tarsus und Fersenbeinhöcker wurden visuell anhand einer vierteiligen Skala bewertet und Wärmebilder wurden mit einer Infrarotkamera (FLIR T440) aufgenommen. Die zweite Studie umfasste ebenfalls eine visuelle Bewertung und darüber hinaus eine histologische Untersuchung von 25 Tarsen und 26 Fersenbeinhöcker von Milchkühen. Die Proben wurden an einem Schlachthof in Österreich gesammelt und die histologische Untersuchung wurde in Zusammenarbeit mit der Universität für Veterinärmedizin in Wien durchgeführt. In der ersten Studie (visuelle Bewertung und thermografische Untersuchung) führte eine Zunahme des Haarverlustes (in Bezug auf die Größe des betroffenen Gebiets) zu einem Anstieg der Hauttemperatur. Dieser Effekt wurde deutlicher an den Tarsen und Fersenbeinhöcker, die zusätzlich eine Ulzeration zeigten. In der zweiten Studie wurden bereits leichte Haarverluste (lockiges Haar, kein Haarausfall) durch entzündliche Prozesse gekennzeichnet. Wobei eine weitere Zunahme des Haarverlustes meist mit einer gesteigerten Entzündungsreaktion einherging. Diese Ergebnisse unterstützen somit unsere Hypothese, dass bereits erste Anzeichen von Haarverlusten am Sprunggelenk durch entzündliche Prozesse gekennzeichnet sind und daher relevant für die Gesundheit und das Wohlbefinden der Milchkuh sind.

10 Acknowledgement

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12 List of figures

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14 Appendix

14.1 Scoring table – Study 1

Date:

Scored by:

	hock	
0 =	hair undisturbed; no hair loss	
1 =	hair disturbed; no hair loss	mild
2 =	hair loss ≤ 2.5 cm in diameter	moderate
3 =	hair loss > 2.5 cm in diameter	severe
	ulceration	
0 =	absent	moderate
1 =	present	severe

	point of the hock
0 =	hair undisturbed; no hair loss
1 =	hair disturbed; no hair loss
2 =	hair loss ≤ 2.5 cm in diameter
3 =	hair loss > 2.5 cm in diameter
	ulceration
0 =	absent
1 =	present

sticker	cow no.	ir - image no.	control	hock right	ulceration right	point of hock right	ulceration right	hock left	ulceration left	point of hock left	ulceration left
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								
			☐								

14.2 Scoring table – Study 2 (before slaughter)

Date:

Scored by:

hock	
0 =	hair undisturbed; no hair loss
1 =	hair disturbed; no hair loss
2 =	hair loss ≤ 2.5 cm in diameter
3 =	hair loss > 2.5 cm in diameter
ulceration	
0 =	present
1 =	absent

point of hock	
0 =	hair undisturbed; no hair loss
1 =	hair disturbed; no hair loss
2 =	hair loss ≤ 2.5 cm in diameter
3 =	hair loss > 2.5 cm in diameter
ulceration	
0 =	present
1 =	absent

sticker	Ohrmarkennummer	Laufnummer	ir – image	hock left	ulceration left	point of hock left	ulceration left

14.3 Scoring table – Study 2 (after slaughter)

Date:

Laufnummer	Ohrmarkennummer	Fellnummer	hock / point of hock	hock score	point of hock score