Effects of subclinical footpad dermatitis and emotional arousal on surface foot temperature recorded with infrared thermography in turkey toms (*Meleagris gallopavo*)

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ABSTRACT Footpad dermatitis is a condition that causes lesions on the plantar surface of the footpads in growing turkeys. Potential inflammatory processes and pain associated with increasing severity of footpad dermatitis raise animal welfare concerns. This study investigated whether the temperature of the plantar surface of the foot (the footpads and the entire plantar foot including interdigital membranes) assessed with infrared thermography reflect severity of mild footpad dermatitis as assessed with a Visual Analogue Scale in 80 turkey toms at 10 weeks of age. In order to study effects of a potential emotional arousal due to the testing procedures, effects of sequential testing order and duration of handling of the turkeys was included in the model. Footpad temperatures were significantly lower than foot temperatures ($p<0.001$, $R^2=0.57$, $-3.36^0C+/-0.28^0C$), and higher visual analogue scale scores were anti-correlated with footpad (-0.06^0C +/- 0.037^0C) and foot temperatures (-0.07^0C +/- 0.066^0C). Furthermore, a negative association between footpad temperature and handling time (-0.02 +/- 0.0227, $p=0.048$), and a non-linear association between foot and footpad temperatures and sequential testing order, was found ($p<0.001$). The results indicate that severity of mild footpad dermatitis as scored visually was associated with the temperatures of the plantar surface of the foot and footpads, and that thermal imaging therefore represent a novel tool for the reliable and non-invasive early detection of subclinical foot pathologies in turkeys. The association was negative, and the findings therefore indicate that potential inflammatory processes in the epidermis at this early stage of footpad dermatitis are negligible, and/or that the hyperkeratosis of the surface keratin shielded heat emission from the footpads. The associations between surface temperatures, handling time and sequential testing order suggest an emotional arousal in response to the experimental procedures, and these factors need to be considered when applying infrared thermography in future studies of leg health in turkeys.

Key words: animal welfare, infrared thermography, leg health, turkey, footpad

INTRODUCTION

The concern for animals' ability to suffer has a long history. Today, citizens worldwide attach a great importance to animal welfare. These welfare discussions also relate to turkeys kept for meat production (Martrenchar, 1999; Anonymus, 2015; Special Eurobarometer 2015). For instance, the prevalence and severity of footpad dermatitis (**FPD**), which is a condition that causes necrotic lesions on the plantar surface of the footpads in growing turkeys, is recognized as an important animal welfare issue (Martland, 1984; Ekstrand and Algers, 1997; Martrenchar, 1999; Clark et al., 2002; Mayne et al., 2006; Shepher and Fairchild, 2010; Krautwald-Junghanns et al., 2011; Bergmann et al., 2013). The cause of FPD is multifactorial, and a wide variety of risk factors including litter quality are identified. Litter quality, in turn, is affected by many other factors related to stocking density, air temperature and humidity, season, consistency and amount of faeces affected by diet, high litter moisture and drinker design (Martland, 1984; Mayne, 2005; Mayne et al., 2007a,b; Youssef et al., 2011). The welfare concerns relate to the potential inflammatory processes and pain associated with FPD. Studies found impaired gait and lameness in turkeys suffering from FPD, and behavioral indications of pain relief when given analgesics, which suggest that footpad lesions are painful (Sinclair et al., 2015; Weber Wyneken et al., 2015).

Externally, FPD starts as small areas of skin discolorations that often develop horn-like pegs of abnormal keratin, which progress into cracks and scabs on the footpads, and the footpad can become swollen and splitting. At a cellular level, hyperkeratosis of the surface keratin and epithelial hyperplasia can often be observed along with acute inflammation and necrosis of the epidermis (Greene et al., 1985; Mayne, 2005; Mayne et al., 2006). Such inflammatory processes are also evident in FPD found in broiler chickens (Shepherd and Fairchild, 2010), and could be evident even in milder forms of FPD (Martland, 1984). Even one week old, birds with beginning external signs of FPD (skin surface discoloration) showed abnormal cellular changes of the footpad integument (Mayne et al., 2006). Externally normal footpads may show microscopic evidence of lesions (Mayne et al., 2006), and the correlation between external and histopathological scores can be low (Mayne et al., 2007a,b). These findings raise the concerns that even milder/subclinical forms of FPD in turkeys may be associated with inflammatory processes.

Infrared thermography (**IRT**), also known as thermal- or thermographic imaging, is a noninvasive, quantitative diagnostic tool that involves the detection of infrared radiation (heat) emitted from an object (Speakman and Ward, 1998), and has been applied as a diagnostic tool to identify inflammatory processes, injury and, indirectly, pain in mammals (McCafferty, 2007). For instance, IRT was a useful tool for the early detection of subclinical foot pathologies in dairy cows (Alsaaod and Büscher, 2012) and lameness in horses (Eddy et al., 2001). Thermal imaging has been widely used in avian research (McCafferty, 2013) to investigate e.g. stress and emotions in chickens (e.g. Cabanac and Aizawa, 2000; Edgar et al., 2011; Moe et al., 2012; Herborn et al., 2015; Moe et al., 2017), but only one study reported the use of IRT to study leg pathologies in poultry (Wilcox et al., 2009). They found that plantar foot temperatures increased with increasing severity of foot lesions (bumblefoot) and after inoculation with *Staphyolococcus aureus*, and suggested that thermal imaging may represent a more sensitive indicator of subclinical infections than visually observed macroscopic lesions in laying hen feet. Thus, IRT could potentially represent a novel tool for the reliable and non-invasive early detection of subclinical foot pathologies and, indirectly, inflammatory processes and pain in turkeys. However, the associations between visually observed macroscopic FPD in its milder forms and surface footpad temperatures have, to our knowledge, not been studied in turkeys.

If not recording thermo- images of the animals feet by taking picture of the surface on which the animal/bird has stepped or automatically by placing the camera in a certain spot and taking the picture using remote control, the application of IRT to screen for potential inflammatory processes in turkey footpads under field study conditions implies that the birds are handled for individual thermal recording. It has been well documented that acute physical and psychological stress and emotional arousal due to handling triggers a sympathetically-mediated cutaneous vasoconstriction causing a rapid drop in surface skin temperature. Such decrease is accompanied by an increase in core temperature, and a subsequent vasodilatation in order to dissipate excess heat resulting in a post-stressor increase in surface temperature. This thermoregulatory response is termed stress-induced hyperthermia, psychogenic fever or emotional fever, and has been described in mammalian, avian, reptile and fish species (e.g. Briese and Cabanac, 1991; Cabanac and Gosselin, 1993; Zethof et al., 1994; Cabanac 1999; Cabanac and Aizawa, 2000; Vinkers et al, 2009; Rey et al, 2015). In previous studies in laying hens and broiler chickens, it was found that handling stress affected temperatures of the plantar surface and interdigital membranes (Cabanac and Aizawa, 2000; Herborn et al., 2015; Moe et al., 2017). It could be suggested that experimental procedures involved in thermal imaging of turkey feet (e.g. capture, immobilization, restraint, presence of humans) may be associated with an emotional arousal, thereby affecting surface foot temperatures.

Therefore, in order to gain more knowledge about the use of thermographic imaging in avian medicine in general and studies of leg health in turkeys in particular, the aims of the present study were to 1) investigate the relationship between the temperature of the plantar surface of the foot (i.e. of the footpads and of the entire foot including interdigital membranes) assessed with IRT and the visual scoring of severity of FPD using a Visual Analogue Scale (**VAS**), and 2) investigate effects of sequential testing order and duration of handling of the turkeys. It was hypothesized that the severity of mild subclinical FPD assessed by visual scoring is associated with surface temperatures, and that handling duration and sequential test order negatively affects surface foot temperatures.

MATERIAL AND METHODS

Animals and Husbandry

The experiment was carried out in a commercial Norwegian turkey house (2250 m^2) with artificial lighting, mechanical ventilation and gas and floor heating. The temperature was kept at 17°, and lights were off for eight consecutive hours during night time (23:00-07:00). The turkeys were fed a standard commercial diet (Norgesfôr Råde Mølle) and had free access to water from bell drinkers. The turkeys were housed on concrete floor with wood shavings, and the farmer added fresh wood shavings every week. The toms (n=5600) and hens (n=5300) were kept separately, and toms were allocated 60 % of the area (1350 m^2). (Later, after the hens were slaughtered at 12 weeks, the toms are then given access to the entire area). Maximum animal density in Norway is 38 kg/m^2 when mean live weight is below 7 kg, and 44 kg/m^2 when mean live weight is above 7 kg.

Experimental Procedures

80 male turkeys at 62 days of age were selected by convenience sampling from different locations in the turkey house for visual FPD scoring, followed by IRT recordings of surface foot- and footpad temperatures. Specifically, one experimenter walked slowly towards the turkey flock and manually captured one turkey at a time. The footpads were cleaned with lukewarm water and a sponge and dried with paper towel in order to be able to visually score severity of potential FPD. Under commercial conditions at Norwegian abbatoirs, a 4-point scale is commonly used to score severity of FPD (Norwegian Industry Standards). This 4-point scale scores footpads according to the following category descriptions: 0 – no lesions, 1 – superficial

lesions, each papillae is still visible, 2 – severe lesions with dark colored crusts covering less than 50 % of the footpad and 3 - severe lesions with dark colored crusts covering more than 50 % of the footpad. In the present study, footpads were scored according to a visual analogue scale (VAS) that consisted of a 20 cm horizontal line with separate images from this 4-point scale evenly distributed above the line (Figure 1). Previously, we found a strong association between categorical classifications of FPD severity and this VAS scale (R^2=0.7 $p<0.001$). For each footpad, the scorer visually evaluated the severity of the lesion and placed an X on this line, which later was measured in mm, giving each footpad a two-decimal VAS-score. Two scorers evaluated each footpad and agreed on the VAS score. Finally, the turkey was manually restrained for thermal imaging by a person covered with an aluminium protective shield fitted around the turkey's leg (in order to avoid influences of heat emission from the body of the bird and the person holding the bird). The turkeys were placed in a position where the sternum (keel) was resting on the handlers lap and the head positioned under the handlers left arm. The plantar side of the foot was pointing towards the thermal camera. Birds were released immediately after the thermal image had been taken, and a new bird was immediately enrolled in the study. The time (min) between capture and completed thermal image (handling time), as well as sequential testing order (order of which the turkeys were enrolled in the study) was recorded. The experiment met the guidelines approved by the institutional animal care and use committee (IACUC).

Infrared Thermography

IRT images of the feet were collected with a thermal camera (T620bx, FLIR System AB, Danderyd, Sweden). The thermal camera was placed in front of the birds' right foot at a distance of 25 cm. The camera was set to an emissivity of 0.96, and the ambient temperature of the testing room was maintained at 16,8°C (range 16,7-17,0°C). These values were used to allow correction for environmental changes during image analysis. Image analysis software (FLIR ThermaCAM Researcher) was used to determine the maximum temperature of the digital footpad ("Footpad") and of the plantar side of the entire plantar foot ("Foot") including the interdigital membranes (Figure 2).

Statistical Analyses

All statistical analyses were performed with the free statistical language R (R Development Core Team, 2011). Temperature differences (outcome) between foot and footpad (explanatory variable) were assessed using robust MM-type regression, which has a breakdown point of 50% (Yohai et al., 1991). Statistical associations are only registered if more than 50% of the observations contribute to the trend making the method particularly robust to data of such quality employed in the present paper. Robust regression was also used to examine differences between handling time (explanatory variable) and foot/footpad temperatures (outcome). To determine the relationship between scoring obtained using the VAS scale and temperature we performed robust regression with VAS scale as the response and the foot and footpad temperatures as the explanatory variables. Robust regression was also performed with handling time as the response versus sequential testing order as the explanatory variable. The reliability of the robust regression models were tested by first assessing the model residuals against a scaled normal distribution. Semi-paramteric bootstrap (Canty and Ripley, 2017) was subsequently performed on the estimates from ordinary least squares linear regression models (i.e. robust regression estimates are biased) to substantiate the observed associations. The p-values and estimates presented here were however obtained from the robust regression method as they did not deviate substantially from the bootstrap estimates. Estimates are reported as mean +/- two standard errors (roughly 95% confidence interval assuming an approximate Gaussian distribution) and p values below 0.001 are designated as p<0.001. A slight, but significant, negative association was detected between handling time (explorative variable) and footpad temperatures (outcome). Therefore, the regression models including foot and footpad temperatures were all adjusted for handling time (no association was however detected between foot temperatures and handling time (p=0.202)). Foot and footpad temperatures, respectively, were regressed against sequential testing order (explanatory variable) using a generalized additive model (**GAM**) due to explicit non-linear trends (Wood, 2006). All GAM models were adjusted for handling time. A GAM was also employed to regress handling time (response) against sequential testing order (explanatory variable) which were found significant, even when adjusted for foot and footpad temperatures.

RESULTS

Foot and footpad temperatures are presented in Figure 3. Footpad temperatures were significantly lower than foot temperatures (p<0.001, R^2=0.57, -3.36°C+/-0.28°C).

Testing all temperatures against VAS (Figure 4), we found that all were significantly negatively associated (p<0.05): higher VAS scorings appeared to be slightly anti-correlated with footpad (-0.06°C +/- 0.037°C) and foot temperatures (-0.07°C +/- 0.066°C). Hence, larger areas of discoloration as determined by the VAS were significantly associated with the lower foot and footpad temperatures.

A weak, but significant negative association between footpad temperature and handling time was found (-0.02 +/- 0.0227, p=0.048). There was not a significant association between foot temperature and handling time (p=0.202).

A strong non-linear association between foot (edf=7.149, $p<0.001$, $R^2=0.33$) and footpad temperatures (edf=7.734, $p<0.001$; $R^2=0.52$), as respective responses, and sequential testing order, adjusted for handling time, as explanatory variable, was found. From Figure 5 it can be seen that the association for both foot and footpad is negative (i.e. temperature decreases) up until half of the turkeys have been enrolled in the study, before the trend turns positive and finally stabilizes.

DISCUSSION

Briefly, the results indicate that severity of mild FPD as assessed by visual scoring of area of discoloration using a VAS scale were negatively associated with surface plantar foot and footpad temperatures as recorded by IRT. Furthermore, handling time and sequential testing order affected the surface temperature.

The observed skin discoloration of the footpads are consistent with early stages of FPD in turkeys (Greene et al., 1985; Mayne et al., 2006). Most feet were scored around score 1 in the VAS, and no feet were scored according to the most severe degrees of FPD. Therefore, although we do not know the prevalence, the findings indicate that the flock in general had good foot health. These turkeys were 10 weeks of age, whereas others have found more severe lesions starting even at an earlier age in turkeys (Mayne et al., 2006; Mayne et al., 2007a,b). The relationship between severity of FPD (area of skin discoloration) and surface temperatures found here indicates the potential of IRT to detect subtle differences in mild FPD in turkeys with a high precision. Since the association was negative, the findings indicate that potential inflammatory processes in the epidermis at this early stage of FPD in turkeys may be negligible. Other studies showed that beginning external signs of FPD (surface skin discoloration) were associated with abnormal cellular changes of the footpad integument (Mayne et al., 2006).

However, such potentially associated cellular changes reflecting beginning inflammatory processes could not be identified by a temperature rise in the present study. The results stand in contrast to findings by Wilcox et al. (2009) who found that increasing severity of bumble foot in laying hens, and an experimentally induced *Staphyolococcus aureus* infection of their plantar feet, resulted in increasing surface temperatures as assessed by IRT. It could be suggested that bumblefoot in these adult laying hens (60 weeks of age) had led to more severe inflammatory processes than early FPL in turkeys (10 weeks of age) as identified here. Furthermore, the bumblefoot lesions were apparently more severe in the laying hen study (Wilcox et al., 2009), since they scored bumblefoot as “pustules and swellings visible at the first glance, and any foot that looked red, sligthly swollen and scabbed”. In contrast, only minor spots of surface discolorations were studied here (Figure 1). It could be speculated that these initial signs of FPD detected here (spots of surface skin discoloration) were associated with an initial ischemic necrosis as described in early stages of FPD in other bird species (AZA, 2015). An ischemic necrosis could result in an initial temperature drop due to reduced blood circulation of the plantar surface in early stages of FPD. On the other hand, the results may also indicate that the hyperkeratosis of the surface keratin actually shielded heat emission from potential inflammatory processes of the footpads. Indeed, hyperkeratosis of the surface keratin can often be observed along with acute inflammation of the epidermis (Mayne, 2005; Mayne et al., 2006). Footpad temperatures were lower than foot temperatures (Figure 3), which may indicate that the thicker layer of keratin of the footpads as opposed to thinner skin of the interdigital membrane shielded heat radiation.

The continuous VAS developed for this study was based on the commercially used categorical scale, and was developed in order to explore subtle differences in mild forms of FPD as studied here. We previously found a strong association between outcomes in the VAS and this categorical scale (R^2=0.7, p<0.001; unpublished). The same two observers agreed on the VAS score, but a further validation of the VAS scale for the scoring of turkey FPD is necessary for future studies. Based on the association between outcomes in the VAS score and temperature, it can be concluded that the VAS was useful to score subtle differences in severity of FPL in turkeys with a high precision.

Interestingly, and in agreement with previous studies in broiler chickens (Moe et al., 2017), handling time and sequential testing order affected foot and footpad temperatures (Figure 5). Indeed, foot and footpad temperatures decreased until half of the turkeys had been selected after which the temperatures increased slightly and finally stabilized. It has been well documented that acute psychological stress and emotional arousal initially triggers a sympathetically mediated cutaneous vasoconstriction (i.e. drop in cutaneous temperature) followed by a subsequent vasodilatation resulting in a post-stressor rise in peripheral temperature, also in poultry (Cabanac and Aizawa, 2000; Edgar et al., 2011; Moe et al, 2012; Herborn et al., 2015; Moe et al., 2017). The initial temperature drop and later temperature increase found here (Figure 5) may therefore reflect that turkeys were emotionally aroused and displayed emotional fever or stress-induced hyperthermia during the course of the test situation. We suggest that human presence during the test period and catching process affected surface foot temperatures. All turkeys had visual contact with the experimenters throughout the experiment, because the experimental pen was set up in the part of the turkey barn where the male turkeys were kept. Furthermore, one experimenter walked slowly towards and within the turkey flock and manually captured one turkey at a time, implicating that the last half of the selected turkeys had been exposed to more catching related disturbances compared to the first half. Thus, in agreement with previous studies (Cabanac, 1999; Cabanac and Aizawa, 2000; Edgar et al., 2011; Herborn et al., 2015; Moe et al., 2017), these findings may indicate an emotional origin of the temperature alterations due to handling and sequential test order as found here. However, further studies are needed to confirm the emotional origin of the temperature alterations found here. For instance, it would be necessary to record associated temperature alterations indicative of emotional fever or stress-induced hyperthermia (e.g. core temperature and head/comb surface temperatures) to draw firm conclusions.

In this field study, efforts were made to select the birds as randomly as possible from various locations of the turkey house by convenience sampling. However, since FPD may be associated with pain and lameness (Sinclair et al., 2015; Weber Wyneken et al., 2015) it could be that lame birds and/or turkeys with more sever FPD were easier to capture due to impaired walking ability, which may have confounded the study. However, this may not have been the case, since lameness was not observed (unpublished) and the majority of turkeys displayed only mild forms of FPD. Another confounding factor may be that more fearful birds moved more quickly from the person who sampled the birds and therefore were not included in the study. Since stress and fear may be associated with emotional fever or stress-induced hyperthermia as discussed above, it cannot be ruled out that individual differences in fear towards humans may have affected the temperatures recorded.

In conclusion, IRT represents a novel tool for the reliable and non-invasive early detection of subclinical leg pathologies in turkeys. As the association was negative, the results may indicate that the inflammatory processes in the epidermis at this early stage of FPD in turkeys is negligible, and/or that heat emission from potential inflammatory processes in the footpads the hyperkeratosis were shielded e.g. by surface keratin. It would be interesting to perform histology to investigate potential inflammatory processes in footpads with these milder forms of FPD to verify the hypothesis. Furthermore, experiments are needed to investigate surface temperatures associated with the whole scale of severity of FPD. The results clearly demonstrate that a standardization of protocols is a necessary basis for IRT studies of leg health abnormalities in turkeys, as has been emphasized in IRT studies in humane medicine (e.g. Lahiri et al., 2012). In particular, a precise definition of anatomical region of interest as well as a potential emotional arousal due to e.g. handling time and sequential testing order need to be taken into account in future studies in turkeys using infrared technology.

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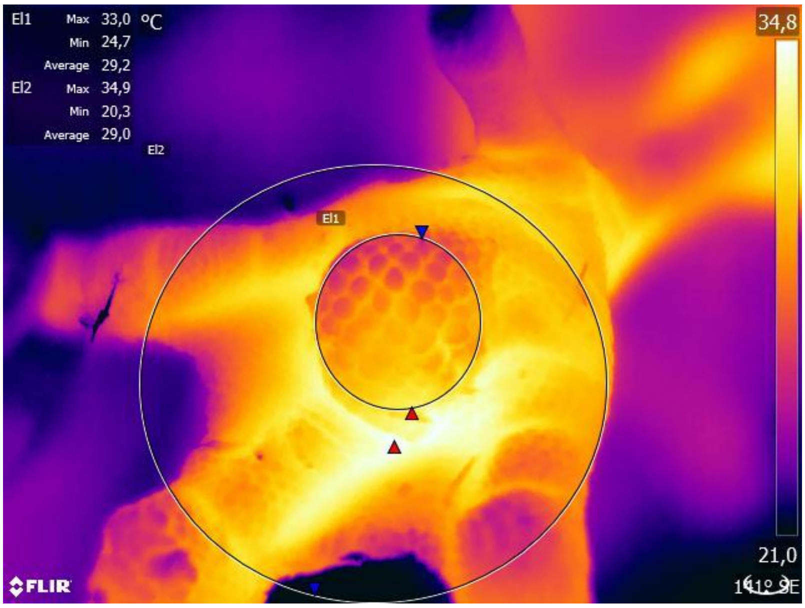
Figures

Figure 1. The Visual Analogue Scale (VAS).

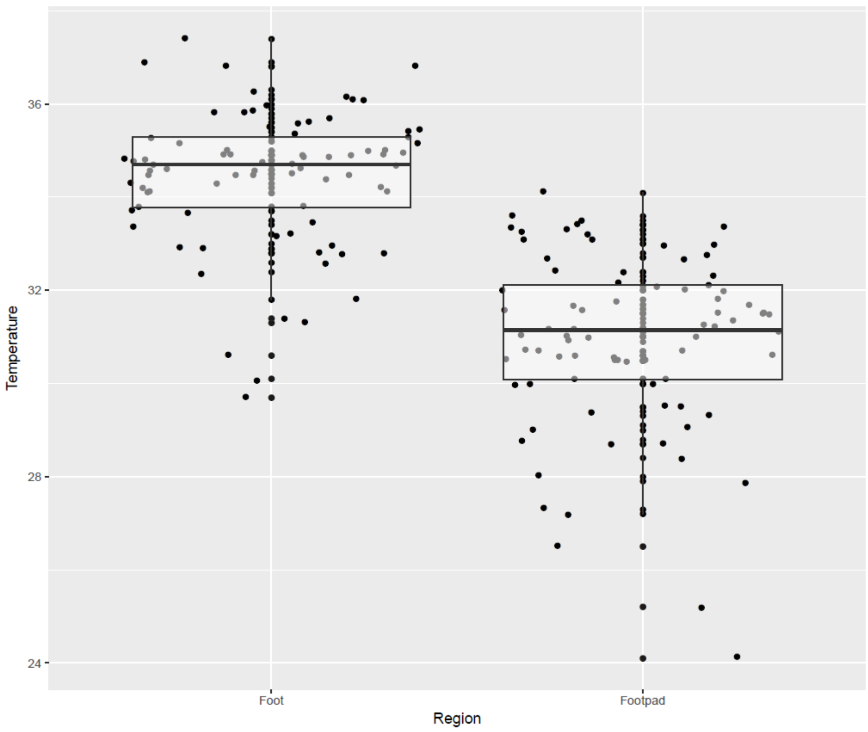
The VAS used for the scoring of severity of footpad lesions, based on the categorical assessment of footpad lesions as used by the Norwegian poultry industry (Norwegian Industry Guidelines).

Figure 2. Plantar foot regions assessed.



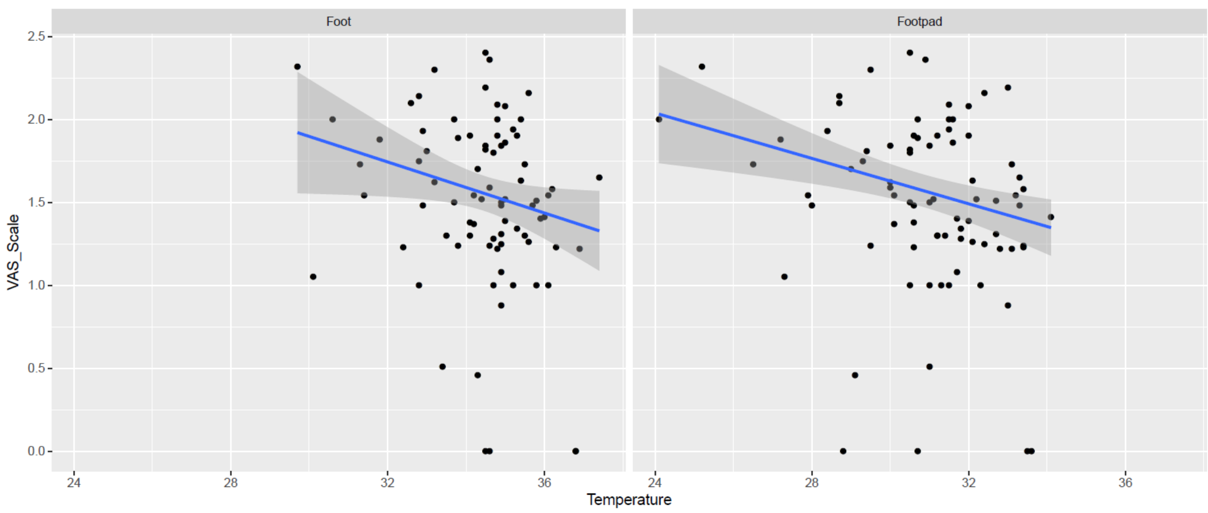
Footpad, and the entire plantar foot including the interdigital membranes.

Figure 3. Foot versus footpad temperatures



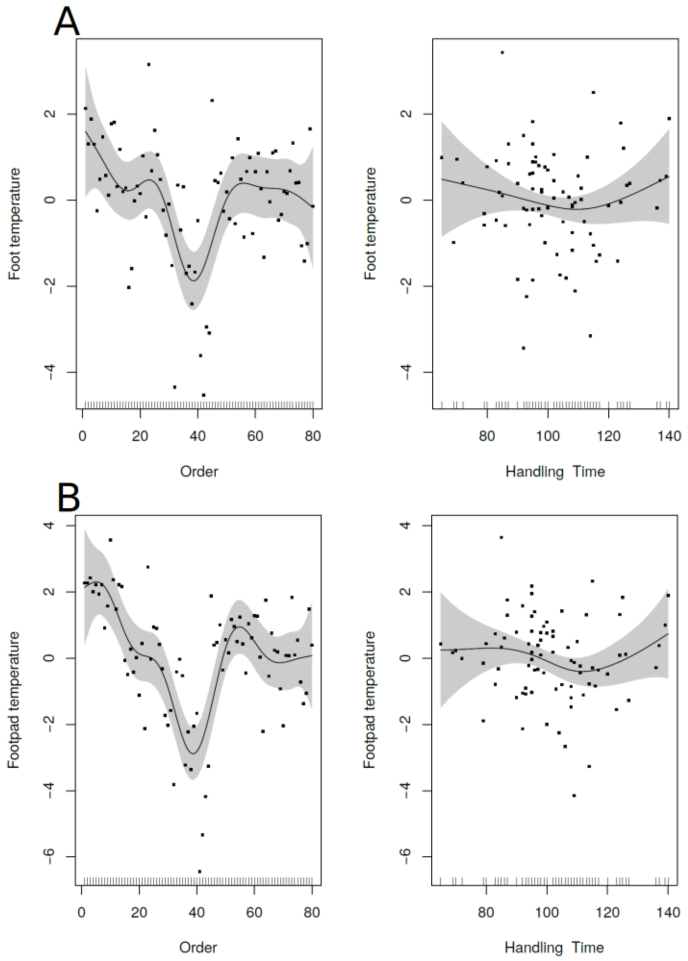
The figure shows a boxplot of foot and footpad temperatures (vertical axis).

Figure 4. Visual Analogue Scale scoring versus foot and footpad temperatures recorded with a thermal camera.



The figure designates foot (left) and footpad (right) temperatures plotted against VAS Scale (vertical axis). The blue trend line is based on a robust regression model.

Figure 5. GAM regression of foot and footpad temperatures versus testing order.



The figure shows foot (A) and footpad (B) mean subtracted temperatures (vertical axis), together with the GAM model fit, plotted against sequential testing order (vertical axis, left panel) and the adjusted covariate handling time (vertical axis, right panel).

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