Experimental factors affecting the within- and between-individual variation of plantar foot surface temperatures in turkeys (*Meleagris gallopovo*) recorded with infrared thermography

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Abstract:

Footpad dermatitis is a welfare concern in turkeys kept for meat production. In order to develop the basis for future standardized infrared thermography (IRT) protocols to screen for impaired foot health, this study investigated within- and between-individual temperature variation in two plantar sub-regions (Footpad, and the whole plantar Foot surface), and effects of cleaning procedures, in 80 turkey toms. A thermal camera (FLIR System AB) was used to collect IRT images. Feet were cleaned with water and dried with a paper towel. The minimum and maximum temperature ($Temp_{min}$ and $Temp_{max}$) of Footpad and Foot in dirty and cleaned feet were determined. Sources of variation related to anatomical region, cleaning procedure and image analysis method were identified. $Temp_{max}$ Foot was significantly higher than $Temp_{max}$ Footpad both before (4.8℃ 95%CI (4.36, 5.19), t=22.9, p<0.001) and after cleaning (3.5℃ 95%CI (2.96, 4.04), t=12.9, p<0.001). Furthermore, $Temp_{max}$ Foot (3.92℃ 95%CI (3.54, 4.3), t=20.6, p<0.001) and $Temp_{max}$ Footpad (2.64℃ 95%CI (2.08, 3.2), t=9.3, p<0.001) were significantly higher before than after cleaning. Potential effects of e.g. evaporation and skin emissivity due to residual water, and shielding properties of dirt are discussed. In general, $Temp_{max}$ variance differences were lower before cleaning than $Temp_{min}$ variance differences. The variance differences between $Temp_{max}$ and $Temp_{min}$ Footpad before cleaning were lower for $Temp_{max}$ (F=3.38, p < 0.001), and $Temp_{max}$ Footpad did not exhibit any significant variance differences before and after cleaning (F=0.75, p=0.2). Thus, it is necessary to create a strict protocol (i.e. specifically define the anatomical region of interest, take into account image analysis methods and cleaning procedures) for reducing errors of temperature measurements in future studies of turkey foot health. Specifically, the results indicate that Footpad $Temp_{max}$, regardless of cleaning procedures, represent an optimal anatomical region and analysis method for future studies where severity of footpad lesions and impact on animal welfare are studied.

Key words: thermal imaging, infrared thermography, turkey, footpad, animal welfare

HIGHLIGHTS:

We investigated sources of variation in surface foot temperatures in turkeys

Anatomical region, cleaning and image analysis method affected temperature

A standardized protocol is necessary for future IRT studies of turkey foot health

1. Introduction

Infrared thermography (IRT), also known as thermal- or thermographic imaging, is a noninvasive, quantitative diagnostic tool that involves the precise measurement of infrared radiation (heat) emitted from an object [1]. The method has been widely applied in biomedical, medical and veterinary studies. For instance, IRT has been used to study skin temperature alterations that may reflect the presence of various pathological conditions, clinical abnormalities and inflammation in underlying tissues, or where blood flow is altered due to stress and emotional arousal in humans [2] and a wide range of mammalian species [3]. Infrared thermography has been suggested to represent a non-invasive tool to study various aspects of animal welfare relevant issues [4]. For instance, previous studies reported on the use of IRT for the early detection of painful leg or hoof problems in horses [5] and cattle [6]. IRT has been widely used in avian research [7] to study heat radiation associated with emotional arousal and stress in e.g. the domestic fowl [8-12].

Footpad dermatitis (FPD) is a welfare concern in growing turkeys worldwide [13-20] due to the potential pain involved, as suggested by evidence of associated inflammatory processes, necrosis, lameness and pain [17, 21-24]. Externally, even normal footpads may show microscopic evidence of inflammatory processes [17], and a link between macroscopic and microscopic features of FPD in broiler chickens was demonstrated [25]. Furthermore, IRT identified subclinical footpad infections ("bumble foot") in laying hens with a higher precision than visual observation [26], suggesting that IRT may be a useful tool for the screening of foot health also in other avian species. Recently, we found that severity of mild footpad dermatitis as scored visually was negatively associated with the temperatures of the plantar surface of the foot and footpads in turkeys [27]. However, studies of leg pathologies in turkeys using IRT are scarce at present.

In general, body temperature may show substantial within- and between-individual variation, and studies from human medicine emphasized the general lack of information about environmental, individual and technical factors influencing the use of IRT [28]. For instance, variation of plantar foot thermographic patterns in healthy humans were identified [29], and an influence of anatomical regions of interest (e.g. different shape and size of region) on diagnostic accuracy of thermal imaging was suggested [30]. They emphasized that a standardization of protocols and selection of regions of interest are essential when applying IRT. However, information on sources of variation of thermographic patterns related to anatomical region of plantar feet in turkeys is currently lacking. Furthermore, as the plantar feet in live turkeys may be covered with debris (i.e. various amounts of faeces, bedding/litter material), it is necessary to clean the plantar foot surface before the visual inspection of FPD. This may in particular be the case under on-farm conditions and in field studies where several factors may affect the measured temperatures. For instance, it was observed that dirt and foreign material on animals may alter emissivity and conductivity (i.e. physical properties of the external surface regarding its effectiveness in emitting energy as thermal radiation, and the property of a material to conduct heat), and excess moisture increased heat loss [31], thus representing important sources of variation in surface temperatures. However, effects of debris and cleaning procedures on thermal radiation from the surface of the plantar foot in turkeys have not been described.

Therefore, in order to develop the basis for future standardized IRT protocols to screen for foot health in turkeys on farm, the aim of this study was to investigate sources of variation in surface plantar foot temperatures. Specifically, within- and between-individual plantar foot surface temperature variation in two plantar sub-regions (footpad, and the whole plantar foot surface including interdigital membranes) and effects of cleaning were investigated.

2. Materials and methods

This study was conducted as part of a larger study which aimed to generate knowledge about the use of thermal imaging in avian medicine in general and studies of leg health in turkeys in particular [27].

2.1. Animals and husbandry

A description of the animals, housing and experimental design is provided in Moe et al. [27]. Briefly, this study was carried out in a commercial Norwegian turkey house (2250 m^2) where the toms (n=5600) and hens (n=5300) were kept separately. The house had artificial lighting (dark between 23:00-07:00), mechanical ventilation and floor heating. The temperature was kept at 17°C, and the turkeys were housed on concrete floor with wood shavings. The birds were fed a standard commercial diet (Norgesfôr Råde Mølle) and had free access to water from bell drinkers.

2.2. Experimental Procedures

Eighty male turkeys at 10 weeks of age were used in this study. The birds were captured individually for visual FPD scoring followed by IRT recordings of surface foot- and footpad temperatures. One of the authors (ECS) walked slowly towards the turkey flock and manually captured one turkey at a time. In order to be able to visually score the severity of potential FPD, the footpads were cleaned with lukewarm water and a sponge and dried with a paper towel. The turkey was then manually restrained for thermal imaging and placed in a position where the sternum (keel) was resting on the handlers lap, the head was positioned under the handlers left arm and the plantar side of the foot was pointing towards the thermal camera. In order to avoid influences of heat emission from the body of the bird and the person holding the bird, the handler was covered with an aluminium protective shield fitted around the turkey's leg. After the thermal image had been recorded, the bird was released immediately and a new bird was enrolled in the study. The experiment met the guidelines approved by the institutional animal care and use committee (IACUC).

2.3. Infrared thermography

A thermal camera (T620bx, FLIR System AB, Danderyd, Sweden) was used to collect IRT images of the feet. The birds' right foot were scanned from a distance of 25 cm. The camera was set to an emissivity of 0.96 and the ambient temperature of the testing arena was maintained at 16.8°C (range 16.7-17.0°C), allowing correction for environmental changes during image analysis. The minimum and maximum temperature ($Temp_{min}$ and $Temp_{max}$) of the digital footpad ("Footpad") and of the plantar side of the entire plantar foot ("Foot") including the interdigital membranes in dirty and cleaned feet (Figure 1a, b) were determined using image analysis software (FLIR ThermaCAM Researcher).

2.4. Statistical methods

To assess differences between Foot and Footpad temperatures, before and after cleaning, we employed Welch's T-test and estimates are given as mean XX ^{0}C together with 95% confidence intervals (CI). The distribution of the temperatures were assessed using histograms and qq-plot and were in all cases found to follow symmetric t-like distributions. Testing for variance differences was carried out using the F – test. All statistical testing and related figures were carried out using the free statistical software R [32] and the package 'ggplot2' [33].

3. Results

Examples of thermal images of a cleaned and not cleaned turkey foot, depicting the anatomical regions that were assessed, are shown in Figure 1. $Temp_{max}$ Footpad and $Temp_{max}$ Foot before and after cleaning are presented in Figures 2 and 3, respectively.

3.1. Region of interest:

$Temp_{max}$ Foot was significantly higher than $Temp_{max}$ Footpad both before (4.8℃ 95%CI (4.36, 5.19), $t=22.9$, $p<0.001$) and after cleaning (3.5℃ 95%CI (2.96, 4.04), $t=12.9$, $p<0.001$).

3.2. Effects of cleaning:

$Temp_{max}$ Foot (3.92℃ 95%CI (3.54, 4.3), $t=20.6$, $p<0.001$) and $Temp_{max}$ Footpad (2.64℃ 95%CI (2.08, 3.2), $t=9.3$, $p<0.001$) were found to be significantly higher before than after cleaning. $Temp_{min}$ Footpad was on the other hand significantly higher after cleaning than before (1.28℃ 95%CI (0.45, 2.11), $t=-3.06$, $p<0.001$).

3.3. Temperature variance:

In general, $Temp_{max}$ variance differences were lower before cleaning than $Temp_{min}$ variance differences. Before cleaning, the variance differences between $Temp_{max}$ and $Temp_{min}$ Footpad were found to be significantly lower for $Temp_{max}$ ($F=3.38$, $p < 0.001$). However, after cleaning, variance differences between $Temp_{max}$ and $Temp_{min}$ Footpad were not statistical significant ($F=1.28$, $p = 0.268$). $Temp_{max}$ Footpad did not exhibit any significant variance differences before and after cleaning ($F=0.75$, $p=0.2$) while $Temp_{min}$ Footpad variances were found to be significantly lower after cleaning ($F=1.97$, $p=0.003$).

Before cleaning, $Temp_{max}$ Foot variances were significant lower ($F=0.32$, $p<0.001$) than after cleaning. We further examined variance differences between $Temp_{max}$ Foot and Footpad before and after cleaning and found that $Temp_{max}$ Foot exhibited significantly lower variance than $Temp_{max}$ Footpad before ($F=3.9$, $p<0.001$) and after cleaning ($F=1.69$, $p=0.02$).

4. Discussion

Overall, the results demonstrated a substantial within- and between individual variations of foot temperatures in turkeys attributed to cleaning procedures, anatomical regions of interest and image analysis method.

4.1. Anatomical region of interest

$Temp_{max}$ for the entire plantar Foot was higher than $Temp_{max}$ of the Footpads, and the difference was evident before and after cleaning. We suggest that these differences can be explained by shielding properties of the thicker layers of keratin of Footpads in contrast to more radiation from thinner skin of the interdigital membranes of the Foot. The findings highlight the importance to clearly define anatomical region of interest for IRT protocols, as emphasized recently [28, 30].

4.2. Temperature in relation to cleaning

In accordance with Palmer [31], temperature shielding properties of dirt was evident to some extent, as indicated by that the $Temp_{min}$ Footpad was significantly higher after cleaning than before (Figure 2), and the lower temperature variance differences in clean Footpads. However, a significant drop in $Temp_{max}$ of Footpads and Foot was found after washing (Figure 2 and 3). Although the feet were dried thoroughly with a dry sponge after washing, there may have been residual water left on the feet, which may have resulted in electromagnetic absorption and altered skin emissivity [34]. Thus, ideally, turkey feet should be cleaned to allow for the feet to dry properly some time before thermal imaging. The potential residual water may also have resulted in a temperature drop due to convection-cooling during evaporation. Indeed, moisture increases local heat loss to the environment [31]. Studies suggested that e.g. sweat may have a cooling effect due to evaporation, in addition to acting as a filter for infrared radiation [35, 36]. Thus, if there was residual water left on the feet after cleaning, evaporation may have influenced the IRT results reported here.

The first thermal image (feet not cleaned) was recorded immediately after capture, and the next was recorded after a period of restraint due to the cleaning procedure. Hence, another plausible explanation is that the temperature drop after cleaning the feet could reflect stress or emotional arousal due to handling and restraint in later-sampled individuals. Indeed, a drop in skin temperature due to cutaneous vasoconstriction caused by stress and emotional arousal has been well documented in human stress research [28, 38], and this phenomenon has also been described in poultry species [8-11]. For instance, handling and restraint stress resulted in a drop in plantar surface foot temperatures due to sequential testing order in broiler chickens [12], and the intensity of acute stress may influence surface temperatures in poultry [11]. The influence of duration of handling stress and sequential testing order on surface temperature was also evident in turkeys (as discussed by Moe at al. [27]) and needs to be taken into account in IRT studies in turkeys, in particular when considering to clean the feet some time prior to thermal imaging to avoid shielding properties of cleaning procedures as discussed above.

4.3. Image analysis method

Footpad $Temp_{max}$ showed lower variance differences than $Temp_{min}$. Furthermore, no variance differences before and after cleaning were found for $Temp_{max}$. These results indicate that $Temp_{max}$ gives more precise measurements than $Temp_{min}$ in IRT studies of turkey footpads, regardless of cleaning procedures. This is not surprising, since $Temp_{min}$ measurements may reflect various shielding effects of debris or even “air” (e.g. risk of inaccurate measurements related to the interdigital space, see Figure 1). The findings are in line with Ludwig et al. [37], who found that obtaining a temperature value of a specific area based on $Temp_{max}$ detection shows less operator dependencies and give more meaningful results compared to the average temperature of the same area. As discussed above, area calculation based on Footpads or including a wider area (i.e. Foot) clearly affected the results, in line with studies in humane medicine [28, 30].

5. Conclusion

This study identified several sources of variation affecting the within- and between-individual variation of plantar surface temperatures in turkeys recorded with infrared thermography under field study conditions. Sources for this variation may be attributed to anatomical regions of interest, effects of cleaning procedures, and image analysis method. Furthermore, an emotional origin of the temperature effects was discussed previously [27]. Thus, it is necessary to create a strict protocol (i.e. specifically define the anatomical region of interest, take into account image analysis methods, cleaning procedures and handling time) for reducing errors and increasing the accuracy and the precision of temperature measurements in future studies of turkey foot health. Footpad and $Temp_{max}$ measurements resulted in the most precise measurements regardless of cleaning procedures and therefore may represent an optimal anatomical region and analysis method for future studies and protocols. Further studies on turkey skin emissivity are of particular interest to correct the surface temperature due to changes in emissivity in the case of cleaning procedures.

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Figure 1

Plantar anatomical regions assessed: Footpad (inner circle), and the entire plantar Foot including the interdigital membranes (large circle). The images show a typical thermal image before (a) and after (b) cleaning. The debris and litter material was mostly attached to the Footpad, whereas the interdigital membranes of the Foot were mostly clean.

Fig 1a

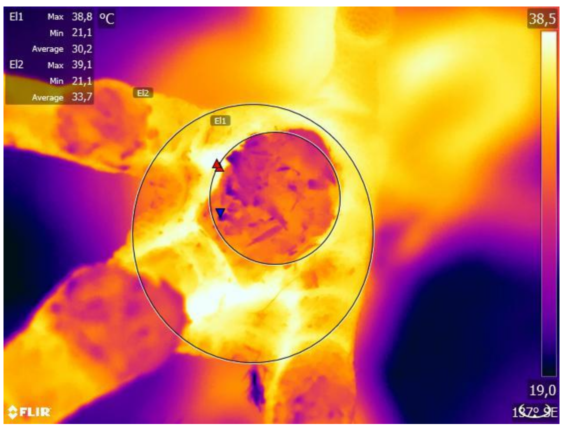


Fig 1b

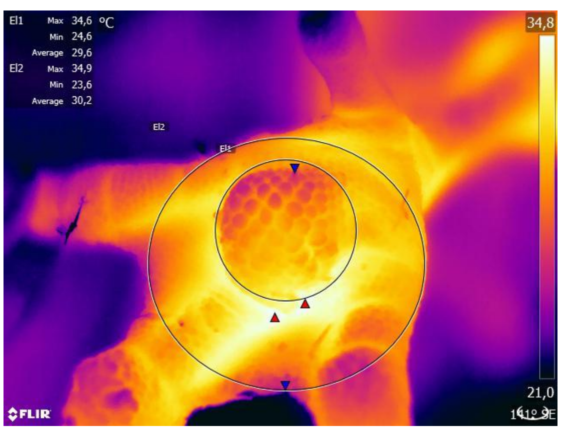


Figure 2

Maximum and minimum surface temperatures (°C) recorded in Footpads, before and after cleaning.

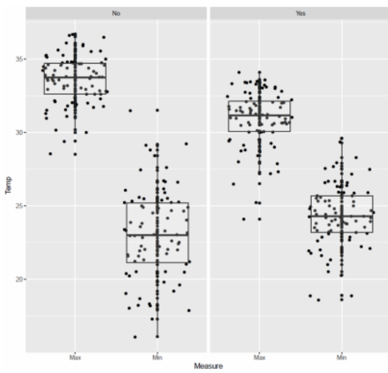
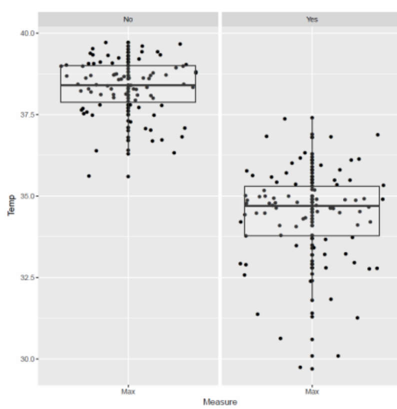


Figure 3

Maximum surface temperatures (°C) recorded in Foot, before and after cleaning



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