

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

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22 Animal Well-Being and Behavior

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23 **ABSTRACT** Footpad dermatitis is a condition that causes lesions on the plantar surface of the
24 footpads in growing turkeys. Potential inflammatory processes and pain associated with
25 increasing severity of footpad dermatitis raise animal welfare concerns. This study investigated
26 whether the temperature of the plantar surface of the foot (the footpads and the entire plantar
27 foot including interdigital membranes) assessed with infrared thermography reflect severity of
28 mild footpad dermatitis as assessed with a Visual Analogue Scale in 80 turkey toms at 10 weeks
29 of age. In order to study effects of a potential emotional arousal due to the testing procedures,
30 effects of sequential testing order and duration of handling of the turkeys was included in the
31 model. Footpad temperatures were significantly lower than foot temperatures ($p < 0.001$,
32 $R^2 = 0.57$, $-3.36^{\circ}\text{C} \pm 0.28^{\circ}\text{C}$), and higher visual analogue scale scores were anti-correlated with
33 footpad ($-0.06^{\circ}\text{C} \pm 0.037^{\circ}\text{C}$) and foot temperatures ($-0.07^{\circ}\text{C} \pm 0.066^{\circ}\text{C}$). Furthermore, a
34 negative association between footpad temperature and handling time (-0.02 ± 0.0227 ,
35 $p = 0.048$), and a non-linear association between foot and footpad temperatures and sequential
36 testing order, was found ($p < 0.001$). The results indicate that severity of mild footpad dermatitis
37 as scored visually was associated with the temperatures of the plantar surface of the foot and
38 footpads, and that thermal imaging therefore represent a novel tool for the reliable and non-
39 invasive early detection of subclinical foot pathologies in turkeys. The association was negative,
40 and the findings therefore indicate that potential inflammatory processes in the epidermis at this
41 early stage of footpad dermatitis are negligible, and/or that the hyperkeratosis of the surface
42 keratin shielded heat emission from the footpads. The associations between surface
43 temperatures, handling time and sequential testing order suggest an emotional arousal in
44 response to the experimental procedures, and these factors need to be considered when applying
45 infrared thermography in future studies of leg health in turkeys.

46

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

48

INTRODUCTION

49 The concern for animals' ability to suffer has a long history. Today, citizens worldwide
50 attach a great importance to animal welfare. These welfare discussions also relate to turkeys
51 kept for meat production (Martrenchar, 1999; Anonymus, 2015; Special Eurobarometer 2015).
52 For instance, the prevalence and severity of footpad dermatitis (**FPD**), which is a condition that
53 causes necrotic lesions on the plantar surface of the footpads in growing turkeys, is recognized
54 as an important animal welfare issue (Martland, 1984; Ekstrand and Algiers, 1997; Martrenchar,
55 1999; Clark et al., 2002; Mayne et al., 2006; Shepher and Fairchild, 2010; Krautwald-Junghanns
56 et al., 2011; Bergmann et al., 2013). The cause of FPD is multifactorial, and a wide variety of
57 risk factors including litter quality are identified. Litter quality, in turn, is affected by many
58 other factors related to stocking density, air temperature and humidity, season, consistency and
59 amount of faeces affected by diet, high litter moisture and drinker design (Martland, 1984;
60 Mayne, 2005; Mayne et al., 2007a,b; Youssef et al., 2011). The welfare concerns relate to the
61 potential inflammatory processes and pain associated with FPD. Studies found impaired gait
62 and lameness in turkeys suffering from FPD, and behavioral indications of pain relief when
63 given analgesics, which suggest that footpad lesions are painful (Sinclair et al., 2015; Weber
64 Wyneken et al., 2015).

65 Externally, FPD starts as small areas of skin discolorations that often develop horn-like
66 pegs of abnormal keratin, which progress into cracks and scabs on the footpads, and the footpad
67 can become swollen and splitting. At a cellular level, hyperkeratosis of the surface keratin and
68 epithelial hyperplasia can often be observed along with acute inflammation and necrosis of the
69 epidermis (Greene et al., 1985; Mayne, 2005; Mayne et al., 2006). Such inflammatory processes
70 are also evident in FPD found in broiler chickens (Shepherd and Fairchild, 2010), and could be
71 evident even in milder forms of FPD (Martland, 1984). Even one week old, birds with beginning
72 external signs of FPD (skin surface discoloration) showed abnormal cellular changes of the

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73 footpad integument (Mayne et al., 2006). Externally normal footpads may show microscopic
74 evidence of lesions (Mayne et al., 2006), and the correlation between external and
75 histopathological scores can be low (Mayne et al., 2007a,b). These findings raise the concerns
76 that even milder/subclinical forms of FPD in turkeys may be associated with inflammatory
77 processes.

78 Infrared thermography (**IRT**), also known as thermal- or thermographic imaging, is a
79 noninvasive, quantitative diagnostic tool that involves the detection of infrared radiation (heat)
80 emitted from an object (Speakman and Ward, 1998), and has been applied as a diagnostic tool
81 to identify inflammatory processes, injury and, indirectly, pain in mammals (McCafferty,
82 2007). For instance, IRT was a useful tool for the early detection of subclinical foot pathologies
83 in dairy cows (Alsaad and Büscher, 2012) and lameness in horses (Eddy et al., 2001). Thermal
84 imaging has been widely used in avian research (McCafferty, 2013) to investigate e.g. stress
85 and emotions in chickens (e.g. Cabanac and Aizawa, 2000; Edgar et al., 2011; Moe et al., 2012;
86 Herborn et al., 2015; Moe et al., 2017), but only one study reported the use of IRT to study leg
87 pathologies in poultry (Wilcox et al., 2009). They found that plantar foot temperatures increased
88 with increasing severity of foot lesions (bumblefoot) and after inoculation with
89 *Staphylococcus aureus*, and suggested that thermal imaging may represent a more sensitive
90 indicator of subclinical infections than visually observed macroscopic lesions in laying hen feet.
91 Thus, IRT could potentially represent a novel tool for the reliable and non-invasive early
92 detection of subclinical foot pathologies and, indirectly, inflammatory processes and pain in
93 turkeys. However, the associations between visually observed macroscopic FPD in its milder
94 forms and surface footpad temperatures have, to our knowledge, not been studied in turkeys.

95 If not recording thermo- images of the animals feet by taking picture of the surface on
96 which the animal/bird has stepped or automatically by placing the camera in a certain spot and
97 taking the picture using remote control, the application of IRT to screen for potential

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98 inflammatory processes in turkey footpads under field study conditions implies that the birds
99 are handled for individual thermal recording. It has been well documented that acute physical
100 and psychological stress and emotional arousal due to handling triggers a sympathetically-
101 mediated cutaneous vasoconstriction causing a rapid drop in surface skin temperature. Such
102 decrease is accompanied by an increase in core temperature, and a subsequent vasodilatation in
103 order to dissipate excess heat resulting in a post-stressor increase in surface temperature. This
104 thermoregulatory response is termed stress-induced hyperthermia, psychogenic fever or
105 emotional fever, and has been described in mammalian, avian, reptile and fish species (e.g.
106 Briese and Cabanac, 1991; Cabanac and Gosselin, 1993; Zethof et al., 1994; Cabanac 1999;
107 Cabanac and Aizawa, 2000; Vinkers et al, 2009; Rey et al, 2015). In previous studies in laying
108 hens and broiler chickens, it was found that handling stress affected temperatures of the plantar
109 surface and interdigital membranes (Cabanac and Aizawa, 2000; Herborn et al., 2015; Moe et
110 al., 2017). It could be suggested that experimental procedures involved in thermal imaging of
111 turkey feet (e.g. capture, immobilization, restraint, presence of humans) may be associated with
112 an emotional arousal, thereby affecting surface foot temperatures.

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

122

123

MATERIAL AND METHODS

124

Animals and Husbandry

126

127 The experiment was carried out in a commercial Norwegian turkey house (2250 m²)
128 with artificial lighting, mechanical ventilation and gas and floor heating. The temperature was
129 kept at 17°, and lights were off for eight consecutive hours during night time (23:00-07:00).
130 The turkeys were fed a standard commercial diet (Norgesfôr Råde Mølle) and had free access
131 to water from bell drinkers. The turkeys were housed on concrete floor with wood shavings,
132 and the farmer added fresh wood shavings every week. The toms (n=5600) and hens (n=5300)
133 were kept separately, and toms were allocated 60 % of the area (1350 m²). (Later, after the hens
134 were slaughtered at 12 weeks, the toms are then given access to the entire area). Maximum
135 animal density in Norway is 38 kg/m² when mean live weight is below 7 kg, and 44 kg/m² when
136 mean live weight is above 7 kg.

137

Experimental Procedures

139

140 80 male turkeys at 62 days of age were selected by convenience sampling from different
141 locations in the turkey house for visual FPD scoring, followed by IRT recordings of surface
142 foot- and footpad temperatures. Specifically, one experimenter walked slowly towards the
143 turkey flock and manually captured one turkey at a time. The footpads were cleaned with
144 lukewarm water and a sponge and dried with paper towel in order to be able to visually score
145 severity of potential FPD. Under commercial conditions at Norwegian abattoirs, a 4-point scale
146 is commonly used to score severity of FPD (Norwegian Industry Standards). This 4-point scale
147 scores footpads according to the following category descriptions: 0 – no lesions, 1 – superficial

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148 lesions, each papillae is still visible, 2 – severe lesions with dark colored crusts covering less
149 than 50 % of the footpad and 3 - severe lesions with dark colored crusts covering more than 50
150 % of the footpad. In the present study, footpads were scored according to a visual analogue
151 scale (VAS) that consisted of a 20 cm horizontal line with separate images from this 4-point
152 scale evenly distributed above the line (Figure 1). Previously, we found a strong association
153 between categorical classifications of FPD severity and this VAS scale ($R^2=0.7$ $p<0.001$). For
154 each footpad, the scorer visually evaluated the severity of the lesion and placed an X on this
155 line, which later was measured in mm, giving each footpad a two-decimal VAS-score. Two
156 scorers evaluated each footpad and agreed on the VAS score. Finally, the turkey was manually
157 restrained for thermal imaging by a person covered with an aluminium protective shield fitted
158 around the turkey's leg (in order to avoid influences of heat emission from the body of the bird
159 and the person holding the bird). The turkeys were placed in a position where the sternum (keel)
160 was resting on the handlers lap and the head positioned under the handlers left arm. The plantar
161 side of the foot was pointing towards the thermal camera. Birds were released immediately after
162 the thermal image had been taken, and a new bird was immediately enrolled in the study. The
163 time (min) between capture and completed thermal image (handling time), as well as sequential
164 testing order (order of which the turkeys were enrolled in the study) was recorded. The
165 experiment met the guidelines approved by the institutional animal care and use committee
166 (IACUC).

167

168 *Infrared Thermography*

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170 IRT images of the feet were collected with a thermal camera (T620bx, FLIR System
171 AB, Danderyd, Sweden). The thermal camera was placed in front of the birds' right foot at a
172 distance of 25 cm. The camera was set to an emissivity of 0.96, and the ambient temperature of

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173 the testing room was maintained at 16,8°C (range 16,7-17,0°C). These values were used to
174 allow correction for environmental changes during image analysis. Image analysis software
175 (FLIR ThermaCAM Researcher) was used to determine the maximum temperature of the digital
176 footpad (“Footpad”) and of the plantar side of the entire plantar foot (“Foot”) including the
177 interdigital membranes (Figure 2).

178

179 *Statistical Analyses*

180

181 All statistical analyses were performed with the free statistical language R (R
182 Development Core Team, 2011). Temperature differences (outcome) between foot and footpad
183 (explanatory variable) were assessed using robust MM-type regression, which has a breakdown
184 point of 50% (Yohai et al., 1991). Statistical associations are only registered if more than 50%
185 of the observations contribute to the trend making the method particularly robust to data of such
186 quality employed in the present paper. Robust regression was also used to examine differences
187 between handling time (explanatory variable) and foot/footpad temperatures (outcome). To
188 determine the relationship between scoring obtained using the VAS scale and temperature we
189 performed robust regression with VAS scale as the response and the foot and footpad
190 temperatures as the explanatory variables. Robust regression was also performed with handling
191 time as the response versus sequential testing order as the explanatory variable. The reliability
192 of the robust regression models were tested by first assessing the model residuals against a
193 scaled normal distribution. Semi-parametric bootstrap (Canty and Ripley, 2017) was
194 subsequently performed on the estimates from ordinary least squares linear regression models
195 (i.e. robust regression estimates are biased) to substantiate the observed associations. The p-
196 values and estimates presented here were however obtained from the robust regression method
197 as they did not deviate substantially from the bootstrap estimates. Estimates are reported as

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198 mean +/- two standard errors (roughly 95% confidence interval assuming an approximate
199 Gaussian distribution) and p values below 0.001 are designated as $p < 0.001$. A slight, but
200 significant, negative association was detected between handling time (explorative variable) and
201 footpad temperatures (outcome). Therefore, the regression models including foot and footpad
202 temperatures were all adjusted for handling time (no association was however detected between
203 foot temperatures and handling time ($p = 0.202$)). Foot and footpad temperatures, respectively,
204 were regressed against sequential testing order (explanatory variable) using a generalized
205 additive model (**GAM**) due to explicit non-linear trends (Wood, 2006). All GAM models were
206 adjusted for handling time. A GAM was also employed to regress handling time (response)
207 against sequential testing order (explanatory variable) which were found significant, even when
208 adjusted for foot and footpad temperatures.

209

210

RESULTS

211

212 Foot and footpad temperatures are presented in Figure 3. Footpad temperatures were
213 significantly lower than foot temperatures ($p < 0.001$, $R^2 = 0.57$, $-3.36^\circ\text{C} \pm 0.28^\circ\text{C}$).

214 Testing all temperatures against VAS (Figure 4), we found that all were significantly negatively
215 associated ($p < 0.05$): higher VAS scorings appeared to be slightly anti-correlated with footpad
216 ($-0.06^\circ\text{C} \pm 0.037^\circ\text{C}$) and foot temperatures ($-0.07^\circ\text{C} \pm 0.066^\circ\text{C}$). Hence, larger areas of
217 discoloration as determined by the VAS were significantly associated with the lower foot and
218 footpad temperatures.

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

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

228

229

DISCUSSION

230

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

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

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

268 The continuous VAS developed for this study was based on the commercially used
269 categorical scale, and was developed in order to explore subtle differences in mild forms of
270 FPD as studied here. We previously found a strong association between outcomes in the VAS
271 and this categorical scale ($R^2=0.7$, $p<0.001$; unpublished). The same two observers agreed on

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272 the VAS score, but a further validation of the VAS scale for the scoring of turkey FPD is
273 necessary for future studies. Based on the association between outcomes in the VAS score and
274 temperature, it can be concluded that the VAS was useful to score subtle differences in severity
275 of FPL in turkeys with a high precision.

276 Interestingly, and in agreement with previous studies in broiler chickens (Moe et al.,
277 2017), handling time and sequential testing order affected foot and footpad temperatures
278 (Figure 5). Indeed, foot and footpad temperatures decreased until half of the turkeys had been
279 selected after which the temperatures increased slightly and finally stabilized. It has been well
280 documented that acute psychological stress and emotional arousal initially triggers a
281 sympathetically mediated cutaneous vasoconstriction (i.e. drop in cutaneous temperature)
282 followed by a subsequent vasodilatation resulting in a post-stressor rise in peripheral
283 temperature, also in poultry (Cabanac and Aizawa, 2000; Edgar et al., 2011; Moe et al, 2012;
284 Herborn et al., 2015; Moe et al., 2017). The initial temperature drop and later temperature
285 increase found here (Figure 5) may therefore reflect that turkeys were emotionally aroused and
286 displayed emotional fever or stress-induced hyperthermia during the course of the test situation.
287 We suggest that human presence during the test period and catching process affected surface
288 foot temperatures. All turkeys had visual contact with the experimenters throughout the
289 experiment, because the experimental pen was set up in the part of the turkey barn where the
290 male turkeys were kept. Furthermore, one experimenter walked slowly towards and within the
291 turkey flock and manually captured one turkey at a time, implicating that the last half of the
292 selected turkeys had been exposed to more catching related disturbances compared to the first
293 half. Thus, in agreement with previous studies (Cabanac, 1999; Cabanac and Aizawa, 2000;
294 Edgar et al., 2011; Herborn et al., 2015; Moe et al., 2017), these findings may indicate an
295 emotional origin of the temperature alterations due to handling and sequential test order as
296 found here. However, further studies are needed to confirm the emotional origin of the

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297 temperature alterations found here. For instance, it would be necessary to record associated
298 temperature alterations indicative of emotional fever or stress-induced hyperthermia (e.g. core
299 temperature and head/comb surface temperatures) to draw firm conclusions.

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

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

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322 a potential emotional arousal due to e.g. handling time and sequential testing order need to be
323 taken into account in future studies in turkeys using infrared technology.

324

325

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326

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331 Agricultural Agreement Research Fund, and Animalia — Norwegian Meat & Poultry Research
332 Centre.

333

334 **Figures**

335

336 **Figure 1.** The Visual Analogue Scale (VAS).

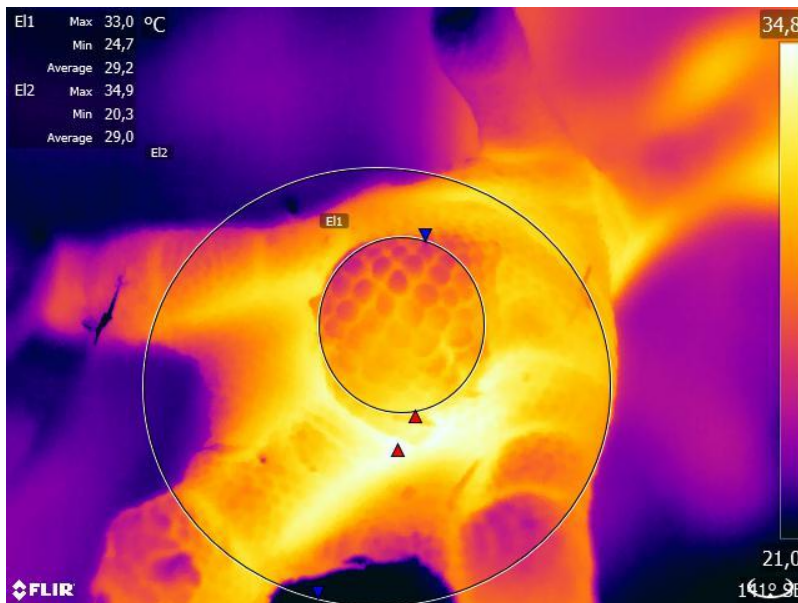


337

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

341

342 **Figure 2.** Plantar foot regions assessed.

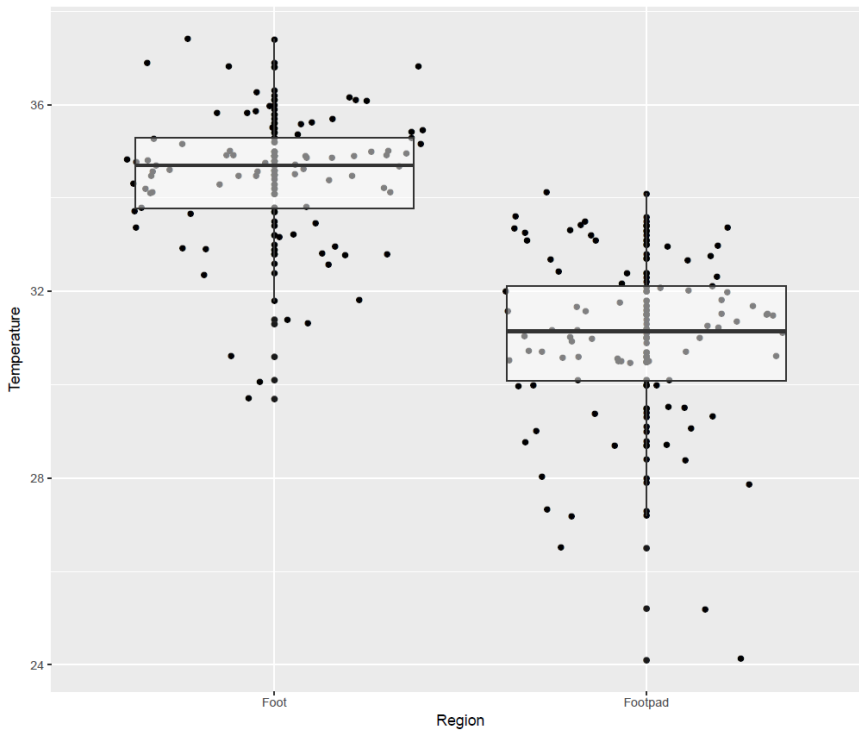


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344 Footpad, and the entire plantar foot including the interdigital membranes.

345

346 **Figure 3.** Foot versus footpad temperatures

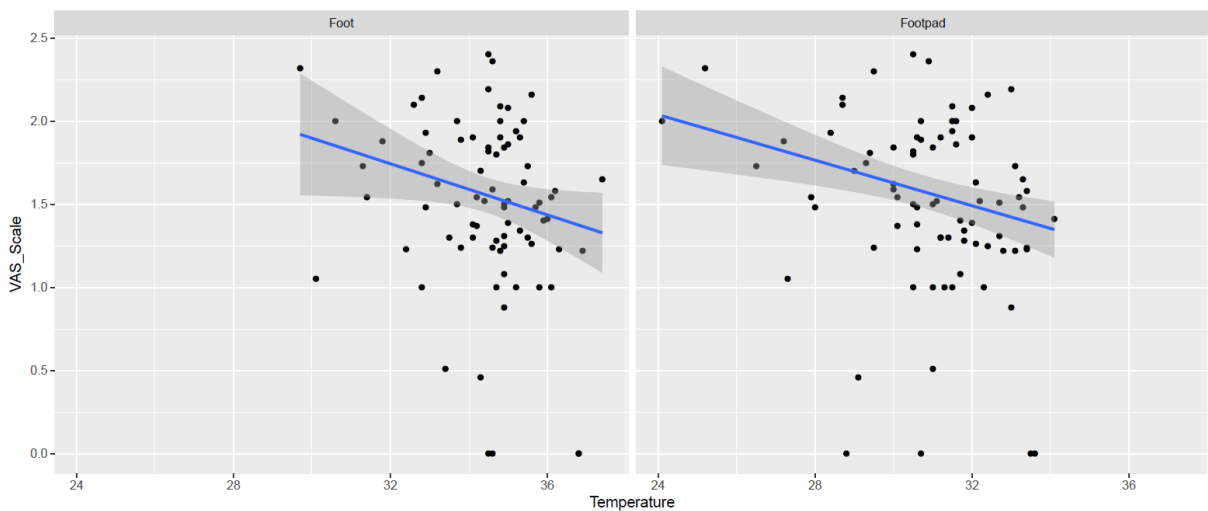


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348 The figure shows a boxplot of foot and footpad temperatures (vertical axis).

349

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

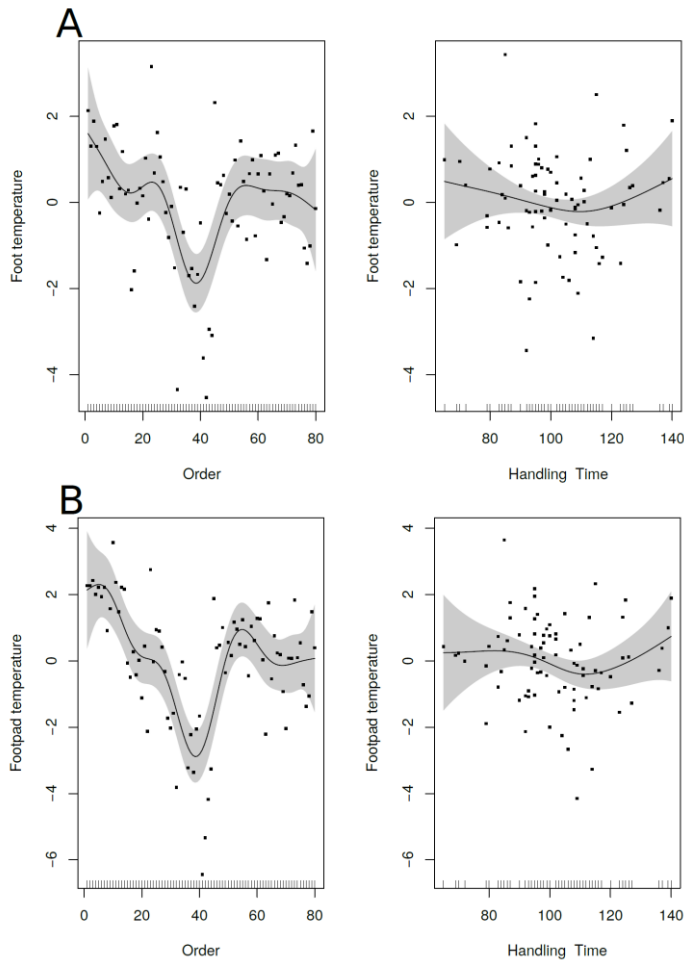


352

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

355

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



357

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

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