Hot chicks, cold feet

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1. Introduction

In recent years, there has been a growing effort to develop scientifically based indicators of emotional states in animals in order to assess their welfare. The subjective components of emotional states cannot be assessed verbally in animals. However, various physiological measurements are used to indirectly detect animal emotions [1,2]. For instance, it has been well documented that acute physical and psychological stress and emotional arousal triggers a sympathetically-mediated cutaneous vasoconstriction causing a rapid drop in skin temperature. This drop is accompanied by a rise in core temperature, followed by a subsequent vasodilatation in order to dissipate excess heat resulting in a post-stressor rise in peripheral temperature. This thermoregulatory response is termed stress-induced hyperthermia, psychogenic fever, or emotional fever, and can be found in mammalian, avian, reptile, and fish species [3-12].

Infrared thermography (IRT), also known as thermal imaging, is a non-invasive, quantitative diagnostic tool that involves the detection of infrared radiation (heat) emitted from an object [13]. Thermal imaging is used in a broad range of animal studies [14], including studies of stress, emotional arousal, and animal welfare in laying hens [15-19]. For instance, handling stress resulted in an initial surface comb and eye temperature drop within a minute of handling by about 2^{0}C and 0.8^{0}C, respectively, whilst core temperature rose over a 9-12 min period in laying hens [8,18,19]. Herborn *et al.* [19] found that the initial stress-induced skin temperature drop (*i.e.* in comb and wattle) was more pronounced and that the post-stressor rise in temperature was largest in response to the most aversive handling procedure, suggesting that stressor intensity can be quantified by measures of skin temperature alterations in laying hens. Previously, we found evidence that a drop in peripheral temperature may reflect the intensity of emotional arousal rather than its valence, as indicated by a drop in surface comb temperature in laying hens during the first minutes of anticipating a palatable food reward [16]. Furthermore, Edgar *et al.* [15] demonstrated that hens respond to an aversive stimulus directed at their chicks by a drop in eye temperature. These studies suggest that a range of head region temperatures may provide valuable information about stress and emotions in poultry.

IRT is useful also for the detection of welfare relevant issues not related to stress and emotions in laying hens. For instance, one study showed a positive relationship between IRT records of surface skin temperature and the visual assessment of plumage condition, which indirectly reflects feather pecking behavior in chicken flocks [20]. Furthermore, IRT was useful for the early detection of subclinical leg pathologies (so-called bumble foot) in laying hens [21].

Taken together, IRT has a great potential to provide valuable information in a variety of animal welfare relevant studies in poultry, ranging from studies of stress and emotions to health related issues [17]. However, although several studies explored temperature in studies of welfare issues in laying hens, less is known about the use of IRT to study welfare in broiler chickens kept for meat production. Leg health problems (e.g. footpad lesions; FPL) are emphasized as important welfare issues in broiler chickens [22], and welfare audits for broilers therefore include the visual inspection of the footpads and scoring of macroscopic appearance of lesion- size and -severity [23]. FPL are associated with inflammatory processes [24,25], which in general are associated with a rise in tissue temperature. Hence, IRT could potentially represent a novel tool for the reliable early detection and/or prediction of subclinical foot pathologies in broiler chickens, as has been suggested for the detection of subclinical bumble foot in laying hens [21].

However, the use of IRT to study footpad temperatures involves handling and restraint of the birds, which may cause stress and emotional arousal, thus having the potential to affect surface temperatures as discussed above. Indeed, foot temperature (in laying hens) may be affected by handling stress: After an initial 6 min drop, the surface temperatures (*i.e.* interdigital membrane temperature read from a digital infrared thermometer) rose [8]. Although one study showed that immobilization of young small broiler chicks resulted in inconsistent and negligible alterations in abdominal skin temperature [26], there is in general limited knowledge about effects of handling and restraint on surface temperatures assessed from IRT in broiler chickens.

Therefore, as a basis for the validation of IRT as a future tool for the early detection and/or prediction of subclinical leg pathologies of broiler footpads, this study investigated effects of factors having the potential to affect surface temperature measurements in clinically healthy broiler chickens associated with the assessment of footpad temperatures. The specific aims were to 1) explore effects of manual restraint on footpad temperatures in broiler chickens; 2) investigate footpad temperatures at two different ages, and 3) explore concomitant effects of manual restraint on several surface head region temperatures, in order to gain more knowledge about effects of stress and emotional arousal on surface skin temperatures in broiler chickens.

2. Material and methods

2.1. Animals and husbandry

The experiment was carried out at the Institute of Production Animal Clinical Sciences at the Norwegian University of Life Sciences. Twenty broiler chickens (Ross 308) were housed in a pen littered with wood shavings. The chickens were obtained from a commercial producer at

15 d of age. The birds had ad-lib access to water from a bell drinker and a commercial diet for broilers (KROMAT Kylling 2, Felleskjøpet, Norway) throughout the experiment.

2.2. Experimental procedures

The birds were accustomed to the housing facilities for 15 d before the start of the experiment. Twelve birds were randomly selected for IRT measurements and tested on three test days during a period of seven days, *i.e.* at 30, 36 (test day 1 and 2; footpad measures) and 37 d of age (test day 3; head region measures). For the footpad measures, each chicken was manually restrained for a total duration of 10 min by a person sitting on a chair. The birds were picked up and gently placed in a position where the ventral side of the feet were pointing upwards towards the thermal camera and with the back leaning against the lap of the handler. The distance between camera and broiler feet was 1m. A cardboard plate covered with aluminium foil to avoid influences of heat emission from the body of the bird and the hands/body of the handler were adjusted and placed on the right leg dorsal to the foot. IRT images of the feet were collected every minute over the 10 min test period (*i.e.* recordings at 0-9 min). For head temperature recordings, birds were gently picked up and manually handled and restrained in the same position as for footpad images. IRT images of the head were collected at the start and the end of a 10 min time period (*i.e.* recordings at 0 and 9 min, then the birds were held in an upright position towards a concrete wall, making sure that the distance from the head to the camera was similar (1 m) for all recordings. The experimenters were located in a corner of the same room as the chicken pen and visible to chickens. Birds were sacrificed after the experiment by blunt trauma and cervical dislocation.

2.3. Infrared thermography

IRT images of the feet and head were collected with a thermal camera (T620bx, FLIR System AB, Danderyd, Sweden). The camera was set to an emissivity of 0.96, and the ambient temperature of the testing room was maintained at 20°C. Relative humidity inside the experimental room was recorded at the beginning and end of every test period. These values were used to allow correction for environmental changes during image analysis. Image analysis software (FLIR ThermaCAM Researcher) was used to determine average surface temperature of the plantar footpad and head (larger anatomical area, see description in Figure 1), and temperatures of the comb base, eye (centre and lateral eye angle), ear, wattles, beak base, and nostril (spot measurements, see description in Figure 2).

2.4. Statistical analysis

Linear mixed effects regression was carried out with footpad temperature as the response variable and time in minutes (duration of manual restraint) as a predictor variable. Additionally, sequential testing order and test day of the experiment (*i.e.* when the chicks were aged 30 and 36 d, respectively), were included as predictors to respectively assess putative effects of waiting time before handling, and of age, on footpad temperature. Individual footpad temperature differences, between the chickens nested within age (*i.e*. test day 1 and 2) with respect to time, were modelled as random slopes:

$$y_{ijkl}=\boldsymbol{X\beta}+\boldsymbol{Zu}+\varepsilon_{ijkl}$$

y_{ijkl} is the foot-temperature response variable with index i=each sample, j=time in min (0-9), k=individual (*i.e.* chick) and l=age (*i.e*. test day 1 or 2). $\boldsymbol{X\beta}$ designates the matrix of fixed effects multiplied with the corresponding parameters to be estimated ($\boldsymbol{\beta}$), while the random effects are represented by the matrix $\boldsymbol{Z}$ multiplied with the corresponding parameters (*i.e.* variances) to be estimated $\boldsymbol{u}$**.**

For control, we fitted another regression model with temperatures, recorded at different head regions (the response variable). Temperatures were collected at two different time points (0 and 10 min after restraint) and this information was included as a predictor variable to be tested. Sequential testing order was included as a predictor but removed since it was not found to be significant (*p=0.66*). The individual variance of each chicken was modelled as a random slope nested for each head region with respect to time point:

$$y_{ijkl}=\boldsymbol{X\beta}+\boldsymbol{Zu}+\varepsilon_{ijkl}$$

y_{ijkl} is the head-temperature response variable with index i=each sample, j=time in min (0 or 9), k=individual (*i.e.* chick) and l=head region (*i.e.* beak, wattle, comb, etc.). Again $\boldsymbol{X\beta}$ designates the matrix of fixed effects multiplied with a parameter vector to be estimated $\boldsymbol{\beta}$, and the random effects are expressed by the matrix $\boldsymbol{Z}$ also multiplied with parameters to be estimated $\boldsymbol{u}$**.** The final model-estimations were carried out using restricted maximum likelihood (REML). Due to the low sample-size we also performed MM-type robust regression [27] with temperature as a response and time point as the explanatory variable for each head region. The quality of the regression models was assessed by examining the residual distribution and by plotting the fitted regression model on the explanatory variables. The Akaike information criterion [28] was used to obtain a quantitative estimate of the model fit. Results from the regression models are reported as mean estimates together with 95% Confidence Intervals (95% CI). The linear mixed effects regression models were fitted using the lme4 package [29]. Degrees of freedom and p-values were computed based on the Satterthwaite method as implemented in the package “lmerTest” [30]. The figures presenting the statistical results were created with the “ggplot2” package [31]. All statistical analyses were performed with the statistical language R [32].

3. Results

One example of a thermal image of a broiler chicken footpad is presented in Figure 1. We found that there was a statistical significant drop ($p<0.001$) in footpad temperature means during restraining of -0.45 ^{0}C 95 % CI (-0.49, -0.41) per minute. Age was also significant ($p<0.001$) in the sense that temperature rose on average with 1.71 ^{0}C 95 % CI (1.04, 2.38) from when the chickens were 30 d of age to 36 d (Figure 3). Sequential testing order of the chickens was also significant ($p=0.04$) for footpad temperatures with 0.13 ^{0}C 95 % CI (0.01, 0.25).

Examples of thermal images of chicken heads are presented in Figure 2. A significant rise in pooled head region temperature means for t=9 as compared to t=0 ($p<0.004$) with 0.76 ^{0}C 95 % CI (0.39, 1.15) was found (Figure 4). We also examined head temperature differences within each specific region of interest using robust regression (Figure 5) and found that only comb base temperature was not statistically significantly different between t=0 and t=9. In all other instances, the temperature rose between the time points. Sequential testing order was not statistically significant for head temperatures ($p=0.66$).

4. Discussion

The present study investigated effects of manual restraint and age on footpad and head region temperatures assessed by thermal imaging in healthy broiler chickens. Briefly, manual restraint resulted in a significant temperature drop in footpads and a temperature rise in the head regions, indicative of the thermoregulatory and vasomotor responses previously described as stress-induced hyperthermia or emotional fever [5,6,10,11]. Furthermore, footpad temperatures differed between the two weeks, where birds at 36 d had higher footpad temperatures than at 30 d.

This study is the first to show that manual restraint results in a significant drop in footpad temperatures in broiler chickens. The results are consistent with previous studies in e.g. laying hens, where handling stress and emotional arousal was associated with an initial drop in surface body temperatures, probably due to skin vasoconstriction during the early minutes of stress and arousal [8,16,18,19]. The results suggest that footpad temperatures drop due to cutaneous vasoconstriction in response to manual restraint. In contrast to previous studies of handling stress and foot temperatures in laying hens, where temperature dropped the initial six minutes before it began to rise [8], the footpad temperatures reported here dropped steadily throughout the immobilization procedure (Figure 3). The results may however not be directly comparable, since Cabanac & Aizawa [8] recorded interdigital membrane temperatures as opposed to footpad temperatures recorded here. However, it seems like the footpad temperature began to rise towards the end of immobilization in some of the chickens (Figure 3). Further studies employing a longer restraint duration would be necessary to investigate at what time point broiler footpad temperature begin to rise after the initial drop.

The order in which the chickens were sequentially sampled affected footpad temperatures: The first chickens restrained had lower temperatures than those restrained last. It could be suggested that human presence during the waiting time and catching process affected thermal responses: All chickens had visual contact with the experimenters throughout the experiment because the pen was in the same room as the experiment was conducted. Furthermore, to capture the chickens, the experimenters entered the pen, which implicated that last chicken caught had been exposed to more catching related disturbances, although none of them showed strong flight responses during capture. This finding may represent a further indication of emotional origin of the temperature alterations found here, and in agreement with studies in group-hosed mice where (colonic) temperature of the last recorded mouse was higher than that of the first mouse in the same cage when recorded sequentially [5]. Thus, duration of manual restraint as well as sequential sampling order need to be taken into account in studies of footpad temperatures in broiler chickens. An effect of sampling order was not found for the head temperature recordings, since head temperatures were only recorded immediately after capture and then after 9 min.

A rise in most of the head region temperatures was found during the restrain period, which may indicate a rise in deep body temperature and subsequent radiation of excess heat during the course of restraint (Figure 4 and 5). This finding is in agreement with several studies of stress and emotions in homeotherms, and hence in support of an emotional origin of the temperature alterations found here [e.g. 6,11]. For instance, both records of eye temperatures (*i.e.* temperature recorded in the center of the eye and in the lateral eye angle) rose during immobilization. This finding is in agreement with Edgar *et al.* [18] who found that even a short period of handling led to a significant rise in eye temperatures. Likewise, a rise in ear and beak base temperature, which are close to the eye region, was detected. Other studies showed an initial drop in eye temperatures during handling indicating vasoconstriction (5 s) before the temperatures rose to levels significantly higher than baseline temperatures to dissipate heat [18,19]. A similar initial drop was not detected here, probably due to the fact that chickens were exposed to capture and handling before the initial temperatures were measured, as opposed to IRT measures of baseline temperatures reported in undisturbed hens [18,19], and no further measurements were undertaken before the last measurement at 9 min. Earlier studies showed that arousal was associated with raised brain temperatures in chickens [33]. Thus, it could be speculated that the close proximity between brain and eye may have influenced eye temperatures recorded here. Furthermore, eye temperature has been suggested to represent a good indicator of core temperature [34]. Although core temperature was not recorded here, it is likely that eye temperature rise indicate a rise in core temperature due to restraint as reported in previous studies [8,18,35]. A rise in nostril temperatures during immobilization may further indicate that core temperature actually had risen, and that excess heat was dissipated by exhalation through the nostrils in addition to a peripheral vasodilatation in the head regions. Furthermore, from the thermal images it was observed that some of the chickens had a slightly open beak at the last recording (9 min), which may indicate that they panted to dissipate heat (see Figure 2b).

The rise in head temperatures during restraint were in agreement with previous studies [18]. The wattles, which together with the comb have an important role in temperature regulation due to their high density of arteriovenous-anastomoses [36-38], showed a rise in temperature due to restraint, in agreement with previous studies [18,19]. However, in contrast to these studies, the comb base temperature was not significantly affected. This lack of effect is most likely a result of the very small size of the comb at this early age and the difficulty to precisely identify the comb base on the thermal image (Figure 2). Thus, studies of stress and emotions in broiler chickens could benefit from replacing spot measures of skin areas of specific interest with average temperatures based on recordings of larger skin areas in the head region. Indeed, head average as well as maximum temperatures (which were recorded on a larger area, in contrast to the specific region spot measurements) were clearly affected by manual restraint, and the individual head region temperatures did not give additional information about emotional arousal during restraint (Figure 5). Therefore, average or maximum head temperature could be employed as a feasible measure of emotional state in future studies of stress and emotions in broiler chickens.

We found evidence that chickens at 36 d consequently had higher footpad temperatures at each recorded point than at 30 days of age (Figure 3). This could be a result of age effects. It was previously found that surface skin temperature measures in the abdominal area drops as a function of age in broiler chickens [39], and the results therefore stand in contrast to previous findings. It is not clear why age affected footpad temperatures. It could be speculated that age dependent anatomical and/or histological alterations, and alterations in circulatory or thermoregulatory capacity due to age, could explain the results. On the other hand, Herborn *et al.* [19] found that the most aversive handling procedures resulted in higher temperatures. Thus, if repeated restraint (*i.e.* measurements week 2) was experienced as more aversive due to a conditioned response than restraint in the first week, then it could be suggested that the results reflect an effect of repeated handling and restraint. Indeed, a study in rats showed that repeated (colonic) temperature measurements resulted in a conditioned temperature rise the second week, whereas a gradual habituation and temperature drop was found at later measurements. On the other hand, rectal temperature in mice handled for rectal temperature measurement and reused after 7 or 14 days did not differ from day 1, implying that mice can be reused in studies of stress-induced hyperthermia [5]. Chickens here served as their own controls, and it is therefore not possible to draw conclusions whether the higher temperatures recorded the second week were an effect of age dependent alterations or conditioning/habituation to the handling procedure.

The effects of handling, restraint and sampling order on footpad temperatures could have clinical relevance in veterinary IRT scanning for footpad lesions. If the magnitude of a temperature rise due to e.g. subclinical lesions is low, and if the duration of veterinary procedures involved is prolonged, it could be that emotion-induced confounding temperature effects in IRT measurements of lesions could affect conclusions of such studies. Clearly, duration of capture and restraint as well as sampling order should be included in future experimental protocols in studies of surface temperature of broiler footpads. Further studies will be needed to address how much of a potential inflammation-induced temperature increase that could theoretically be masked by the emotion-induced cooling of feet.

While the statistical models employed in the present study exhibited a good fit to the data, the number of sample points, especially for the head-temperature measurements, may obscure certain effects due to variance problems associates with low-sample sizes. This was most pronounced with respect to testing order as a weak effect was observed for foot temperatures, but not for head temperatures. On the other hand, the weakness of this effect with regards to footpad temperatures calls for caution, although there are several reasons arguing for such an effect. Nevertheless, our result could indicate interesting avenues for future research on stress and emotions in broiler chickens.

5. Conclusions

This study is the first to demonstrate that footpad temperatures drop whereas head region temperatures rise in response to manual restraint duration in broiler chickens, consistent with body temperatures alterations due to stress and emotional arousal termed stress-induced hyperthermia or emotional fever. Furthermore, footpad temperature differed between 30 and 36 d of age, but it is impossible to draw conclusions whether this effect was caused by age or by previous experience (*i.e.* due to habituation or fear-conditioning). Furthermore, sequential sampling order affected temperature. Thus, one needs to take into account several factors such as the duration of handling and restraint as well as the chickens age, previous experience of the birds and sequential sampling order when using IRT technology in future studies aimed at the early detection and/or prediction of subclinical footpad pathologies in broiler chickens.

Acknowledgements

We thank the staff at the Department of Production Animal Clinical Sciences at the Norwegian University of Life Sciences for taking care of the chickens. Sverre Futsæter is greatly acknowledged for excellent technical assistance with analyzing the thermal images. This project was funded by the Norwegian Research Council [NFR-project no. 234191], the Foundation for Research Levy on Agricultural Products, the Agricultural Agreement Research Fund, and Animalia — Norwegian Meat & Poultry Research Centre. Jon Bohlin was funded by the Norwegian Institute of Public Health.

Figure 1: Thermal image of a footpad

The figure shows one example of a thermal image of one individual broiler chickens' footpad. The circle illustrates the anatomical area that was measured. A circle was created within the software to cover as much as possible of the footpad, without covering areas outside of the footpad.

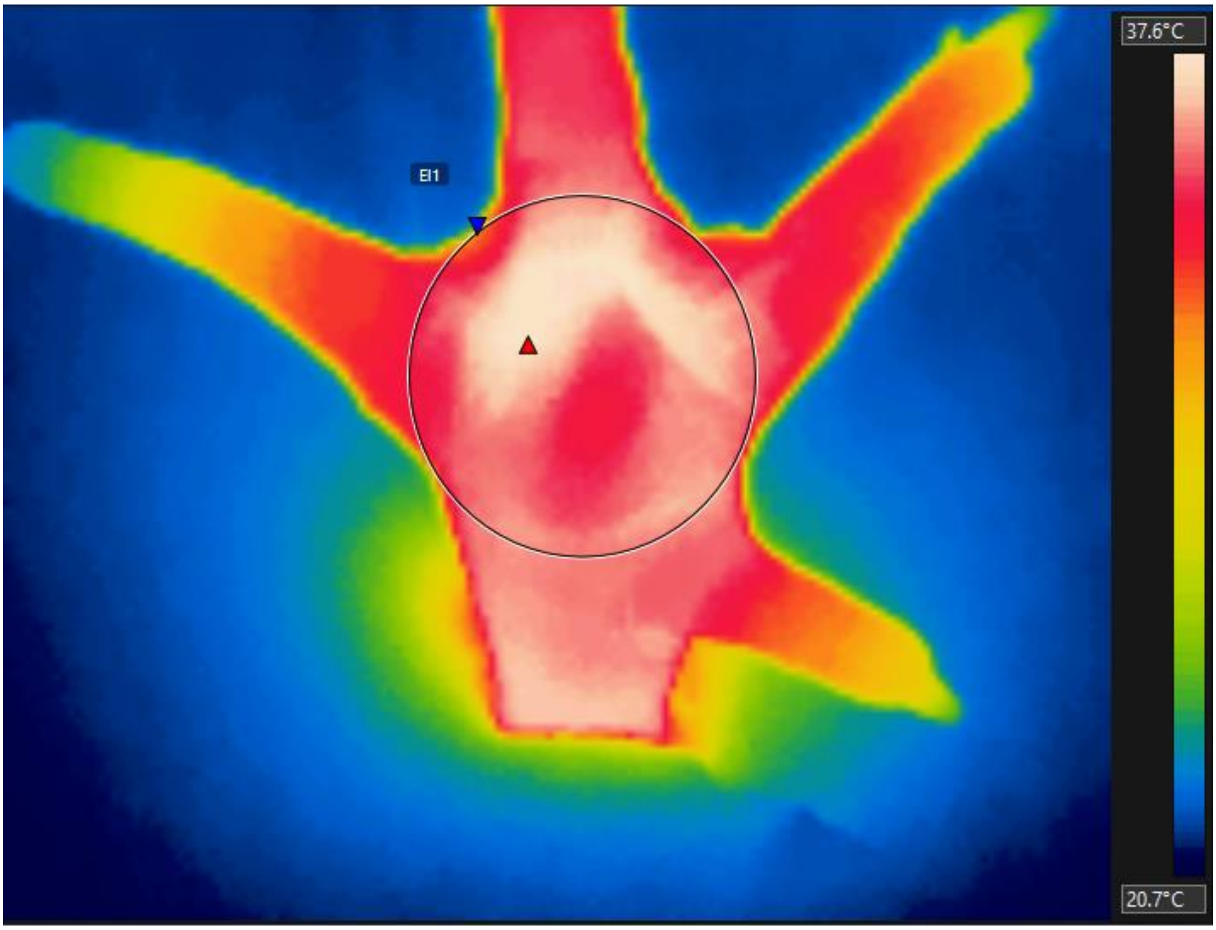
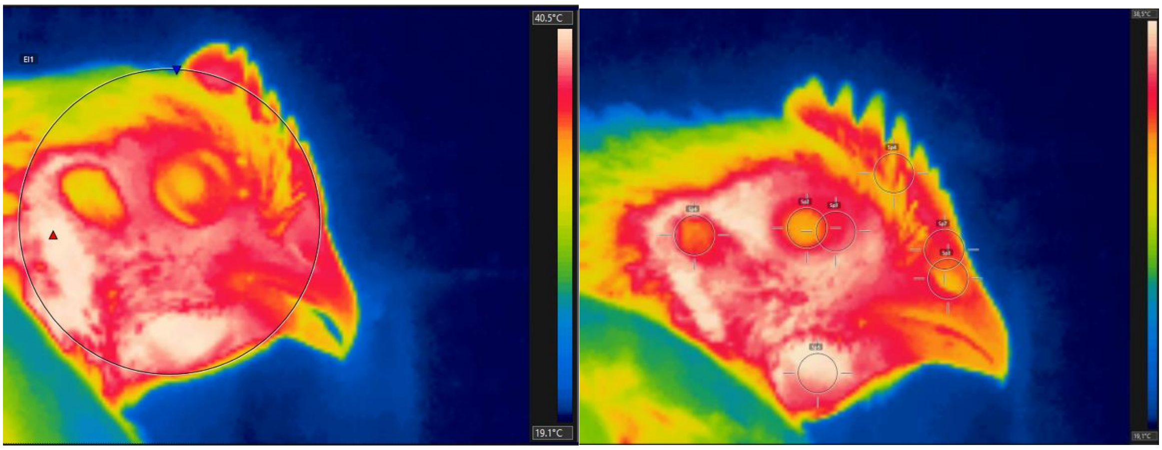


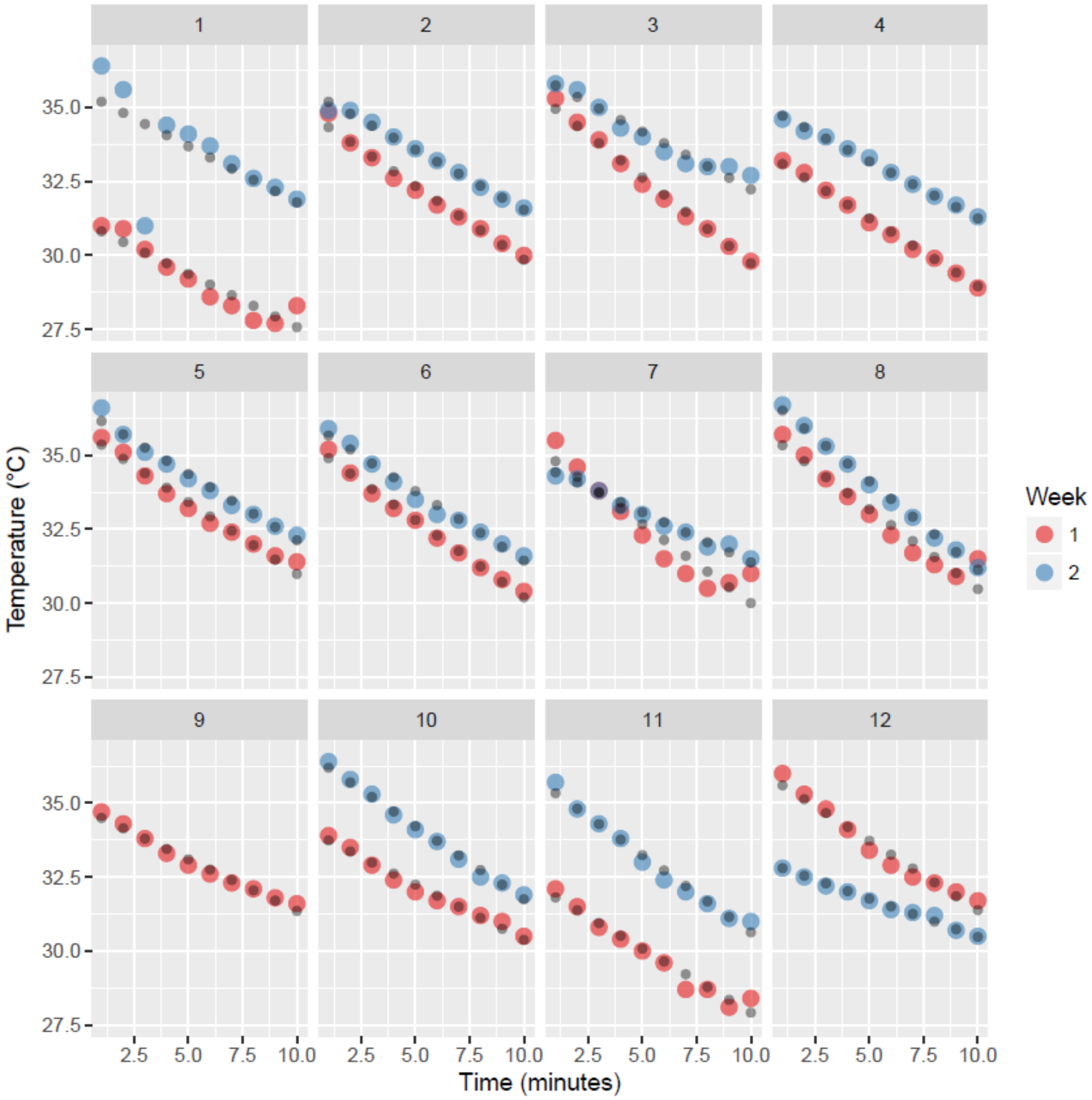
Figure 2: Description of the anatomical location for the different areas and measurement spots included in head temperature recordings.

The figure shows examples of thermal images of broiler chicken heads, and the anatomical location of the areas and measurement spots that were included in the analyses. Figure 2a shows a typical image that depicts the anatomical area where "Head average" and "Head max" temperatures were measured. Here, a circle was created within the software to cover as much as possible of the head, without covering areas outside the head. Figure 2b shows a typical example of the anatomical areas measured as specific measurement spots; Sp1: Eye angle (lateral eye angle, which includes vascularized areas), Sp2: Eye center (middle of the eye), Sp3: Nostril, Sp4: Comb base, Sp5: Wattle, Sp6: Ear, and Sp7: Beak base.



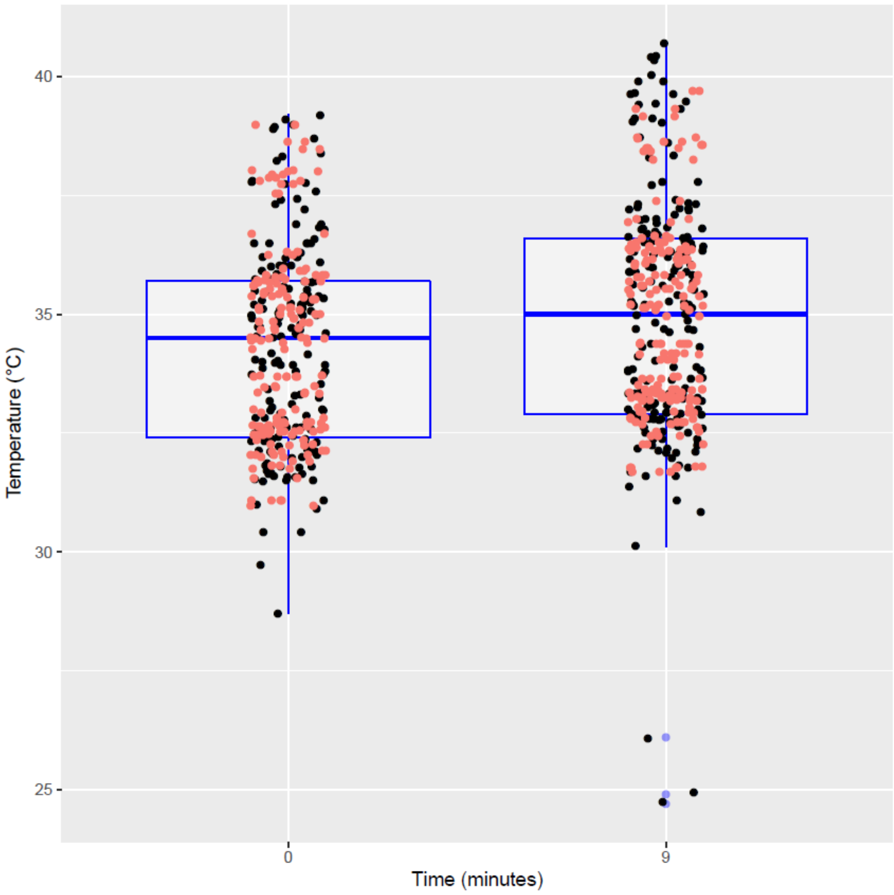
2a **2b**

Figure 3: Footpad temperatures in twelve individual broiler chickens during 10 min. manual restraint, at 30 and 36 d of age.



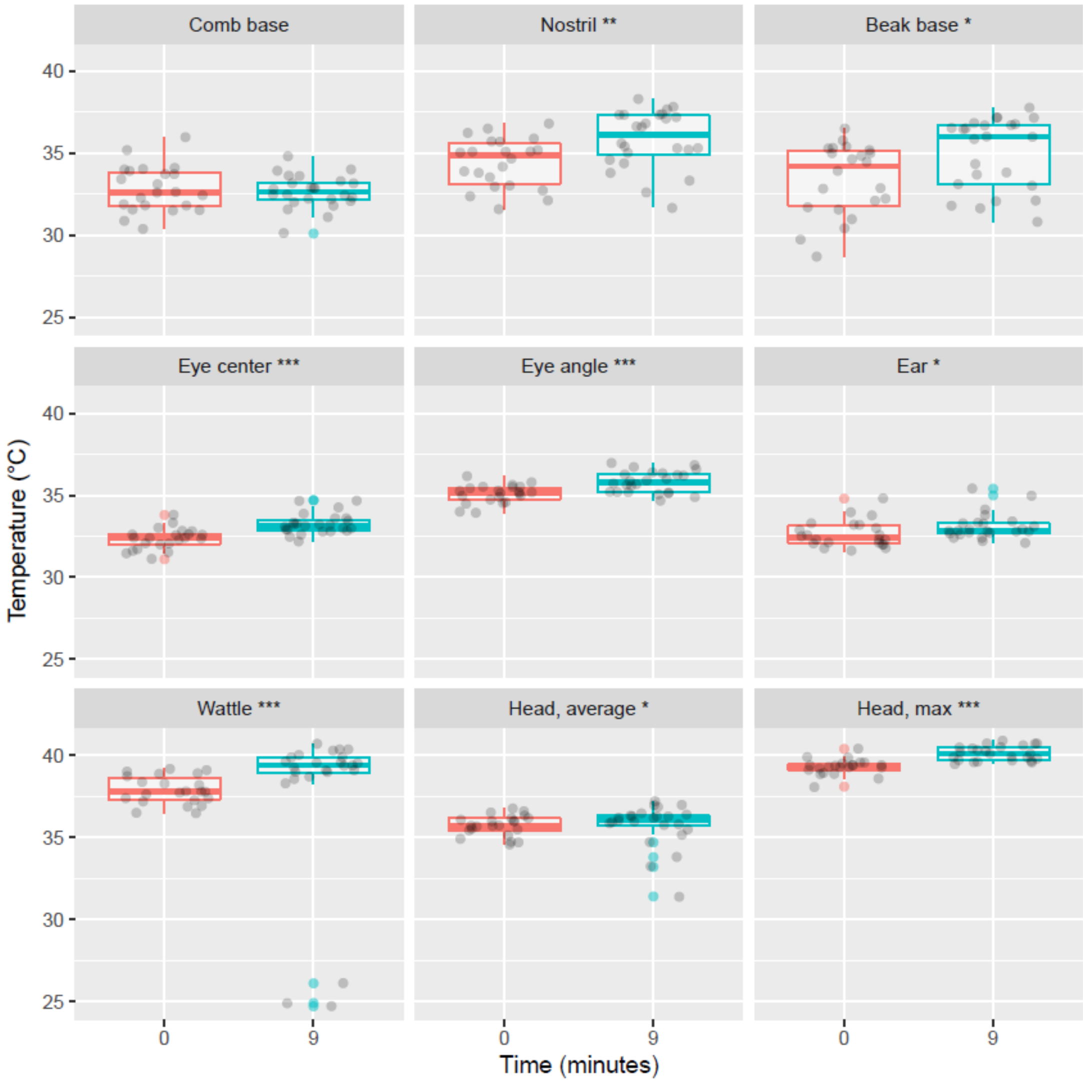
The figure shows recorded footpad temperatures (vertical axis), in degrees of Celsius, plotted against time in minutes (horizontal axis), together with regression model estimates (black points). Each panel, from 1-12 represent each individual chicken with footpad temperatures taken at weeks 1 and 2; *i.e.* 30 and 36 d of age (red and blue lines).

Figure 4: Head region temperatures (pooled) in response to manual restraint.



The figure shows a box plot of pooled head region temperature measurements (black points) plotted against two time points (*i.e.* 0 and 9 min). The red points are temperatures estimated by the mixed effects regression model.

Figure 5: Head region temperatures in response to manual restraint.



The box-plots show head temperature measurements (y-axis) with respect to time (0 and 9 min, x-axis). Each panel corresponds to a separate head region from which temperatures were measured. The stars indicate statistical significance at the $p<0.05$ level (*) $p<0.01$ level (**) and $p<0.001$ level (***).

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