Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/physbeh





Physiology Behavior

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ARTICLE INFO

Keywords: Animal welfare Footpad Infrared thermography Thermal imaging, chicken Stress-induced hyperthermia

ABSTRACT

Infrared thermography (IRT) is increasingly applied as a noninvasive technique for measuring surface body temperature alterations related to e.g. stress, emotions and leg pathologies in avian species. As a basis for the validation of IRT as a future tool for veterinary diagnostics such as detection and/or prediction of subclinical footpad pathologies in broiler chickens, this study explored effects of manual restraint at two different ages on footpad temperatures. Head region temperatures were applied as additional measures of emotional arousal and stress. The study demonstrated that footpad temperatures dropped during 10 min of restraint (p < 0.001, -0.45 °C 95% CI (-0.49, -0.41) per min), whilst head region temperatures (e.g. nostril, wattle, eye, and average head temperature) rose (p = 0.004, 0.76 °C 95% CI (0.39, 1.15) per 10 min), which is consistent with body temperature alterations during emotional arousal and stress, termed stress-induces hyperthermia. Temperature differed between 30 and 36 d (p < 0.001, 1.71 °C 95% CI (1.04, 2.38) per week), but it is impossible to draw conclusions whether this effect was caused by age or by conditioning. Furthermore, sequential sampling order affected temperature (p = 0.04, 0.13 °C 95% CI (0.01, 0.25)). In conclusion, one needs to take into account the duration of handling and restraint during the assessment of footpad temperatures, as well as the chickens age, previous experience and sequential sampling order, when using IRT technology as a future noninvasive tool to study temperature alterations associated with subclinical footpad pathologies in broiler chickens.

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 sympatheticallymediated 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 °C and 0.8 °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

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http://dx.doi.org/10.1016/j.physbeh.2017.05.025

Received 16 March 2017; Received in revised form 25 April 2017; Accepted 18 May 2017 Available online 19 May 2017 0031-9384/ © 2017 Elsevier Inc. All rights reserved.

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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 was 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 1 m. 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 Fig. 1), and temperatures of the comb base, eye (centre and lateral eye angle), ear, wattles, beak base, and nostril (spot measurements, see description in Fig. 2).



Fig. 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.

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