Iyasere OS, Edwards SA, Bateson M, Mitchell M, Guy JH.

Validation of an intramuscularly-implanted microchip and a surface infrared thermometer to estimate core body temperature in broiler chickens exposed to heat stress.

Computers and Electronics in Agriculture 2017, 133, 1-8.

Validation of an intramuscularly-implanted microchip and a surface infrared thermometer to estimate core body temperature in broiler chickens exposed to heat stress

Iyasere, OS*[1], Edwards, SA*, Bateson, M†, Mitchell, M‡ and Guy, JH*

* School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, NE1 7RU, UK;

†Centre for Behaviour and Evolution, Institute of Neuroscience, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK;

‡Animal & Veterinary Sciences, SRUC, Roslin Institute Building, Easter Bush, Midlothian, EH25 9RG, UK;

[1]Corresponding author: o.s.iyasere@gmail.com

Abstract

Heat stress in poultry is associated with an increased core body temperature (CBT) which can be fatal. Methods of estimating CBT range from the minimally invasive method of inserting a digital thermometer into the cloaca of the bird, to the implantation of a temperature recording device (e.g. a data logger) in the peritoneal cavity, a method which gives the most accurate measure of CBT but may be considered invasive. To validate the use of less invasive alternatives to assess CBT in broiler (meat type) chickens using injectable devices, 12 birds were subject to surgery to implant a data logger deep in the body cavity and a microchip in the breast muscle. The birds were then placed in floor pens with an additional 36 birds and subsequently subjected to one of four simulated heat stress conditions over a 3- day period. Measurements of body temperature were collected at intervals from the data logger and microchip (CBT and IM-chip, respectively) along with taken under the wing, feet, comb and cloaca using an infrared thermometer. Changes in body temperature were calculated as the ΔT between pre heat stress and end of 3h heat stress each day. There was no relationship between CBT and IM-chip, but there was a significant correlation between ΔCBT and ΔIM-chip ($R = 0.71$, $P<0.05$) during the heat stress. SBT measured under the wing correlated with CBT ($R = 0.71$, $P<0.05$). Collectively these data confirm the suitability of intramuscularly-implanted microchip and strategically-positioned infrared thermometers to monitor CBT in birds exposed to heat stress, thereby replacing the invasive surgery and associated side-effects of deep body-implanted data loggers for the benefit of animal welfare.

(Key words: broiler chickens, core body temperature, data logger, heat stress, microchip, infrared thermometer)

1. Introduction

A major factor influencing the performance, health and welfare of commercially-reared poultry is the thermal environment to which they are exposed. In the USA, the total annual economic loss due to heat stress in broiler chickens (meat-type birds), layers and turkeys was estimated to be $126.6 million (St-Pierre *et al.*, 2003). Heat stress is also a major cause of the reduced efficiency of poultry production in the tropics (Ojano-Dirain and Waldroup, 2002). During the summer months, birds can be exposed to continuous thermal challenge during heat waves, defined as a period of high temperature and relative humidity (RH) that persists for a minimum of five days (Robinson, 2001). The risk of thermal stress in commercial poultry production is likely to increase with climate change (Skuce *et al.*, 2013). Broiler chickens exposed to high temperature and relative humidity find it difficult to maintain their core body temperature (CBT) (Borges *et al.*, 2007), since unfavourable gradients of temperature and water vapour reduce the opportunity for heat loss through sensible and evaporative means (Widowski, 2010). The resulting increase in CBT (Jensen and Toates, 1997) is a significant welfare issue (DEFRA, 2005). A simple and reliable method for the accurate routine measurement of CBT could therefore be a useful indicator of animal welfare during heat stress.

Measurement of CBT from the brain, viscera and deep skeletal musculature (Schwab and Schafer, 1972) or the hypothalamus, pulmonary artery or oesophagus (Brengelmann, 1987), although accurate (Blainey, 1974), requires a very invasive procedures. Thus, animals must be subjected to general anaesthesia and the placement of the temperature measuring and recording devices involves extensive surgery, Therefore, CBT has typically been measured by placing a digital thermometer into the rectum/cloaca (rectal temperature) of the animal (Quimby *et al.*, 2009). This relatively less invasive procedure (Chen and White, 2006) still has some shortcomings, such as the need to restrain and handle the animal (Torrao *et al.*, 2011), which could result in stress-induced hyperthermia (Dallmann *et al.*, 2006). Although body temperature measured from a thermometer inserted into the colon and abdominally implanted telemetry were positively correlated (R =0.824), temperature of the cloaca was 0.6^{o}C lower than CBT under heat stress conditions (De Basilio *et al.*, 2003). This

difference means that different benchmarks are required regarding assessment of the severity of the heat stress the birds are experiencing.

For continuous, reliable and accurate measurement of CBT, surgically implanted radio-telemetry data loggers (Dawson and Whittow, 2000) and telemetry devices (Lacey *et al*., 2000) have been developed. After suitable calibration against standard thermometers, a major advantage of loggers is that recordings of CBT can be made at pre-set intervals. However, disadvantages of data loggers are risk of infection after invasive surgery, recovery time for the animal prior to commencing any subsequent data collection, the fact that the logger gives only retrospective information about CBT since data can only be accessed after recovery of the logger post-mortem unless telemetric models are used, and finally the possibility of impairing welfare of the bird by obstructing the gastro-intestinal tract or respiratory system (Flecknell and Waterman-Pearson, 2000).

There is an increasing need for accurate measurement and recording of CBT in poultry in controlled studies addressing heat stress and strategies for its alleviation. Development of technologies and sensors that allow measurement and monitoring of CBT in conscious birds with minimal invasion, handling or modification of the animals' behaviour is therefore highly desirable to reduce suffering experienced by animals used in research. Progress has been made internationally in promoting the principles of Replacement, Reduction, and Refinement (the 3Rs) (Medina *et al*., 2015), i.e. where possible to use animal models rather than live animals, and/or reduce the number of animals used, and/or refine the procedures which animals undergo.

Temperature sensing microchips serve the dual purpose of measuring instantaneous body temperature and identification of the animals (Mrozek *et al*., 1995). Microchips require minimally invasive fitting (a simple injection), and typically result in minor tissue injury and discomfort to the birds (Chen and White, 2006), although both site and depth of injection are important considerations (Lohse *et al*., 2010). Identification microchips (RFID, radio frequency identification) with a temperature sensing element operate passively and have no capacity for data storage, but need to be activated by a reader to transmit a radio signal from the scanner to a receiver device (Chen and White, 2006).

Provided these microchips deliver an accurate estimate of CBT, they could serve as a replacement for data logger devices and, because this is a less invasive non-surgical technique, it offers a major refinement to estimating CBT in animals. Whilst the use of microchips to estimate CBT has been validated against other measures of core and rectal temperature in goats (Torrao *et al*., 2011), pigs (Lohse *et al*., 2010) and rabbits (Chen and White, 2006), their use in chickens is yet to be validated.

Another method that might allow estimation of body temperature, and which is fully non-invasive, is by means of an infrared non-contact thermometer to measure surface body temperature (SBT). This approach is dependent on establishing whether a reliable predictive relationship exists between SBT and CBT. Infrared thermometers work by converting radiation emitted from the body into a temperature reading without the need for contact with the animal (Rextroat *et al*., 1999). During heat stress, vasodilation of blood vessels enhances blood flow to the body surface, causing an increase in skin temperature (Yahav *et al*., 2005). In poultry, the less feathered parts of the body (comb, legs, under the wings and the cloaca) (Gerken *et al*., 2006) are mainly involved in heat dissipation.

Given these promising reports of the potential to estimate CBT through implanted microchips and infrared thermometers, the aim of this study was to validate the use of an intramuscularly-injected microchip and a non-contact surface infrared thermometer as refinements of methodologies for the estimation of CBT in broiler chickens exposed to simulated episodic heat stress.

2. Materials and methods

2.1 *Animals*

A total of 48 female broiler chickens of a commercial genotype (Ross 308, Aviagen Ltd, Newbridge, UK; age 26 days, approx. 1100 g) were selected. Following commercial hatchery practices, the birds had previously received IB 4-91 and Hipragumboro vaccines at 7 and 18 days of age respectively. Upon arrival at the experimental facility, the birds were allowed an acclimation period of 7 days, during which temperature and RH of the facility were set at 20^{o}C and 50% respectively, according to recommendations for birds of this age (Aviagen, 1999).

The study was conducted in four identical climate chambers (4.1 x 2.4 x 2.2 m, L×*B*×*H*) equipped with computerized temperature and RH controllers. Birds were subsequently randomly allocated to one of four treatments, one treatment per climate chamber, with three replicate pen groups per treatment (4 birds per pen). Circular floor pens were made from plastic divisions (30 cm high, 93 cm diameter) with wood shavings (Goodwills Ltd, Ponteland, UK) spread 5 cm deep on the floor. Commercial feed (20% crude protein, 4% oil, 6% ash, 13.00MJ ME/kg; W E Jamieson and Son Ltd, Masham, UK) and water were provided *ad libitum* with 6 and 10 cm of feeder and drinker space per bird respectively. Lighting conditions were 16L:8D with an intensity of 30 lux during the light period. At the start of the heat stress treatment, the birds were 46 days old with an average weight of 2423 ± 181.5g.

2.2 Experimental Design

This experiment was conducted under guidelines for animal welfare at both national and local levels. Procedures were approved by the Animal Welfare and Ethics Review Board of Newcastle University and carried out under Project License number PPL 60/4270 of the Animals (Scientific Procedures) Act 1986. To validate methods to estimate CBT in birds exposed to heat stress, it was necessary to simulate moderate episodic heat stress (MEHS) under controlled conditions in the laboratory. The aim was to induce a controlled and modest rise in CBT without resulting in mortality through hyperthermia. The experiment was arranged in a 2×2 factorial design, with two levels of temperature (normal = 20^{o}C and high = 30^{o}C) and two levels of RH (dry = 40% and humid = 70%) to create four different conditions. Each climate chamber contained one particular temperature-RH combination. Thus, depending on the particular treatment, the level of temperature/RH in each climate chamber was gradually increased from 'control' levels (i.e. 20^{o}C and 40% RH) over a period of 1 h (ST; step up in temperature by 2^{o}C every 12 mins; while the RH was increased from 40 % to 70 %) and then held constant for a period of three hours. Once the 3 h had elapsed (3HS), temperature and RH were returned to control levels over a period of 1 h (SD; step down in temperature by 2^{o}C every 12 mins; RH decreased from 70% to 40%).

The pre-heat-stress phase (PrHS) was the period prior to the step up of temperature/RH, and was sufficiently long to allow temperature readings to be taken from all experimental birds (typically 20 mins duration), while the post heat stress period (PHS) was a period of 1 h after the temperature/RH in the chamber had returned to baseline levels.

The experimental protocol involved exposure of the birds to MEHS for a period of 3 h/day for 3 days. A duration of 3h/day of MEHS was considered appropriate since in the UK this would be typical of an elevated heat load endured during either the growing period or during transportation to slaughter (Nicol and Scott, 1990). Similarly 3 days is the average duration of a heat wave (Vale *et al*., 2010).

2.3 Surgery to implant temperature sensors

All the birds in each pen were weighed, and then one bird representative of mean pen bodyweight was selected for surgical implantation of a data logger and concomitant injection of intramuscular microchip. Surgery was necessary to implant the data logger due to its size (external dimensions of 4.0 × 2.7 cm and weight of 20.8 g). Prior to implantation, each logger (Tiny tag, Gemini Talk 2 data logger, Omni Instruments, UK) was calibrated in a water bath and programmed to log CBT data at 3 minute intervals. The loggers all conformed to the manufacturer's specifications, providing absolute accuracy of 0.5°C or better in the specified range (-40 to 85^{o}C) and resolution of 0.05°C or better. The data logger was assembled under aseptic conditions following sterilization of components with ethylene oxide, and then implanted into the abdominal cavity as described below. The birds implanted with a data logger were designated "instrumented birds".

The instrumented birds were injected with a new sterile microchip (identichip® with Bio-Thermo®, Animalcare Limited, York, UK) 3 cm deep into the left breast muscle. This location was chosen to avoid problems of chip migration and environmental influence, whilst not risking harm to the internal organs (Kort *et al*., 1998). The microchip is a passive (battery free) cylinder-shaped device encapsulated in a biocompatible glass capsule and covered with polypropylene cap for anti-migration. The microchip has dimensions of 1.5 x 0.1 cm, weighs 1.0 g and is placed in a needle assembly ready for injection into the animal.

The manufacturer's specification states that the chip can measure temperature with a ±0.5°C accuracy and a resolution of ±0.1°C, within the range of 25-50°C. Both the logger and microchip use thermistor sensors. Subsequent temperature readings were taken by gently restraining the bird and scanning the chip with a hand-held scanner (418-S53-B003-ENG Bio-Thermo® reader, serial number 072942, Digital Angel Corp, MN, USA) within a distance of 5cm.

2.3.1 Surgical procedure

On the day of surgery, feed was withdrawn from the birds 2 h before procedures began. All the birds were weighed individually and legs rings fitted to allow for individual identification. Mean bodyweight was 1445.8 ± 14.3 g. In the theatre, each bird received 2 mg/kg i/m of butorphanol (Pfizer Ltd, Kent, UK) and 1mg/kg i/m of midazolam (Hameln Pharmaceuticals Ltd, Gloucester, UK) to provide pre-emptive analgesia and sedation. General anaesthesia was subsequently induced by administration of 8% sevoflurane in oxygen at a flow rate of 1.5 litre /min through an open flow face mask. Satisfactory induction of anaesthesia was confirmed by the abolition of withdrawal reflexes to comb and toe pinches, attenuation of corneal and nictitating membrane reflexes, and reduction in the pupillary response to light. In addition, 2 mg/kg s/c of Carprofen (Pfizer Ltd, Kent, UK) was administered to provide post-operative analgesia, and 10 mg/kg i/m of enrofloxacin (Bayer Plc, Newbury, UK) to prevent wound infection. Feathers were removed from the sterna carina, and then the bird was placed in dorsal recumbency on a heated table and covered with an insulating blanket.

A 50 mm midline incision was made just below the sternal carina (keel bone), then the duodenum and pancreas were gently lifted out of the abdomen and carefully placed on a saline- soaked piece of cotton wool. The data logger was inserted into the abdomen and gently manipulated so that it was positioned behind the abdominal viscera. The duodenum and pancreas were repositioned, and the peritoneum and abdominal muscle closed with PDS (3-0) interrupted sutures (Johnson & Johnson Medical Ltd, Livingston, UK). The skin was closed using PDS (4-0) using a subcuticular continuous suture (Johnson & Johnson Ltd). Finally, tissue glue was applied to the skin margins.

Next, the microchip was injected 3 cm deep into the left breast muscle using the needle applicator provided by the manufacturer and the bird then placed in an incubator (20^{o}C) under observation until recovery (typically 20-30 mins later). Figure 1 shows the location of the 'data logger' and 'microchip' devices in the chicken. Some 4 h after surgery, each bird was given a second intramuscular injection of enrofloxacin (0.6 ml) as a further precaution against infection.

The 12 birds that underwent surgery were monitored overnight, and their health checked again the following morning before they were returned to their home pens. Recovery of birds was monitored through close daily observation and mean weekly bodyweight gain. The weights of the birds were monitored on the 3rd and 10th day post-surgery, and the weight gain compared with control birds. By the 10th day post- surgery, the bodyweight gain of all 12 sensor birds was comparable with that of control birds and, coupled with observations of apparently normal feeding, drinking and activity, these signs were considered indicative of recovery from surgery. The other 36 birds were not implanted with either data logger or microchip, but were required in this study for collection of further comparative data such as respiratory rate, behaviour, growth performance, feed and water intake (results from these data are not included in this publication).

3. Data collection

3.1 Core and surface body temperature

CBT was automatically measured by the data logger every 3 minutes. Subsequently, the mean of five data points closest to time of reading IM-chip temperature and SBTs were used to derive a comparable mean CBT. Typically it took approximately 15 minutes to determine IM-chip and SBTs from the three instrumented birds in each treatment. To measure IM-chip and SBTs, the bird was picked up from its pen and the microchip scanned by passing the hand-held scanner device across the breast of the bird. Then SBTs under wing, under the feet, on the comb and on the cloaca were estimated using a pre- calibrated infrared thermometer probe (ThermoWorks TW2 mini pocket infrared thermometer, Lindon, USA). The infrared surface temperature probe was calibrated using an ice bath, which involves filling a large glass to the very top with crushed ice, then slowly adding cold water until it reaches about 1 cm below the top of the ice. The mixture was gently stirred and allowed to sit for 2 minutes. Infra-red thermometer set at an emissivity of 0.95 was held such that the

lens was directly above and perpendicular to the surface of the ice bath, the reading on the thermometer was 0.0ºC. The infrared thermometer has an accuracy of ±2% of reading or 2ºC, whichever is greater, resolution of 0.1ºC, wavelength of 5-14 µm, distance: spot ratio of 6:1 and was set at an emissivity of 0.95.

Due to the wide variation observed between birds in baseline IM-chip (e.g. a range of 1.2 ºC; minimum of 40.73 and maximum of 41.93ºC, in PrHS values, Figure 2), temperature estimates were standardized by subtracting the values of body temperature in the PrHS phase from that of subsequent phases. Such standardization gave values for ΔCBT and ΔIM-chip, respectively for the different phases of the heat stress protocol.

Scanning the chip and measuring SBTs took less than two minutes per bird and was carried out at the end of each of the five phases on the 1st and 3rd days of heat stress. At the end of the study, following euthanasia by cervical dislocation and post- mortem examination, the data loggers were recovered and CBT data downloaded onto a PC. All the microchips were recovered from the same site as their implantation.

3.2 Statistical Analyses

Temperatures and ΔTs measured by the data logger, microchip and infrared thermometer were analysed using a repeated measures General Linear Model (GLM) with a full factorial model. The within subject factor was phase of the heat stress protocol, while the between subject factors were temperature and RH. If the test assumption of sphericity was violated (Mauchly's test, $P<0.05$), then the degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity, using SPSS (version 19, SPSS, Rothampstead, UK). The level of statistical significance chosen as the threshold was $P<0.05$.

The relationships between CBT, IM-chip and SBTs, as well as those between their Δ values were analysed using Pearson's correlation and simple linear regression analyses. Further validation of the relationship between ΔCBT and ΔIM-chip was determined using a Bland-Altman plot (Bland and Altman, 1986), an analysis adopted within the medical profession to compare two methods of measurement in order to determine whether the two methods could be used interchangeably or whether the new method could replace an established one (Myles

and Cui, 2007).

4. Results

4.1 Estimates of core body temperature from chip and logger

Although all 12 microchips functioned appropriately to produce barcode and temperature readings, two of the data loggers malfunctioned so that data could not be retrieved from them (one each from the 20°C, 40% RH and the 30°C, 40% RH treatments). Hence data for 10 data loggers are reported. A plot of normal diurnal rhythm of CBT of the logger birds exposed to the different temperature/RH levels for a 3 h period is presented in Figure 2. Since there was no significant interaction between day × temperature × RH on CBT estimated at the end of 3 h of heat stress, subsequent analysis was based on the mean of CBT for 2 days (Days 1 and 3) since day was considered of little importance.

Phase of the heat stress protocol had a significant effect on CBT ($F_{4, 24} = 14.69$, $P<0.001$) and IM-chip ($F_{4, 32} = 5.82$, $P<0.05$). The mean CBT was significantly greater at the end of 3HS & SD phases than in the PrHS, ST& PHS phases (Figure 3). Mean IM-chip was significantly greater at the end of the 3HS than in the PrHS, ST and PHS phases with temperature at the end of SD being intermediate. There was a significant phase × temperature interaction for CBT ($F_{4, 24} = 5.35$, $P<0.05$). Post hoc comparisons showed that at the end of 3HS birds kept at 30°C had a higher CBT than those kept at 20°C. There were no significant interactions of phase × RH or phase × temperature × RH for CBT or IM-chip.

Phase of the heat stress protocol had a significant effect on ΔCBT ($F_{3, 18} = 18.35$, $P<0.001$) and ΔIM-chip ($F_{3, 24} = 5.82$, $P<0.05$). The ΔCBT was significantly greater at the 3HS and SD phases than at the PrHS, end of ST or end of PHS phases (Figure 4). There was a significant phase × temperature interaction for ΔCBT ($F_{3, 18} = 5.63$, $P<0.05$), specifically at the end of 3HS ($P<0.05$) where birds exposed to 30°C had a greater ΔCBT than birds kept at 20°C.

At the end of the 3HS phase, the ΔCBT and ΔIM-chip were 0.7°C and 0.5°C respectively in birds exposed to 30°C and 70% RH. There were no significant phase × RH or phase × temperature × RH interactions for ΔCBT or ΔIM-chip.

4.2 Relationship between core and surface body temperatures

Table 1 shows the correlations between various estimates of CBT, IM-chip and SBT. At the end of 3HS, there was no significant correlation between CBT-logger and IM-chip. However, there were significant correlations between CBT and under wing temperature (WT), between wing and comb temperatures, between wing and feet temperatures and between comb and feet temperatures. In addition, ΔCBT was correlated with ΔIM-chip, under wing and feet temperatures. The ΔIM-chip was correlated with ΔWT. Finally, there was a correlation between ΔWT and Δ feet temperatures (see Table 2). The significant linear regression equations for the prediction of CBT- logger from WT (Equation 1); ΔCBT-logger from ΔWT (Equation 2) and finally ΔIM-chip from ΔWT (Equation 3) are presented below. Equation 1 shows that 51% of the variation in CBT measured by the data logger could be explained by under wing SBT, whilst even more of the variation in ΔCBT measured by the data logger or the chip could be explained by ΔWT.

$CBT = 36.63 + 0.14\ WT\ (R^2 = 0.51, P < 0.05)$ ……..Equation 1

$\Delta CBT = 0.08 + 0.17\ \Delta WT\ (R^2 = 0.75, P < 0.05)$……Equation 2

$\Delta IM\text{-}chip = -0.002 + 0.157\ \Delta WT\ (R^2 = 0.67, P < 0.05)$……Equation 3

4.3 Agreement between the microchip and the data logger in measuring ΔCBT

A Bland Altman plot shows the mean of the differences in temperature between CBT and IM-chip and the limit of agreement between them (mean difference ± 1.96 SD). The mean difference between ΔIM-chip and ΔCBT (known as the bias) was -0.1 ± 0.25 (mean ± SD) which means that the mean ΔIM-chip was 0.1^oC less than ΔCBT, shown as the solid horizontal line in Figure 5.

5. Discussion

The present study investigated the potential use of new minimally invasive methods for the estimation of CBT in broiler chickens by evaluating their performance against a proven but invasive methodology for the continuous measurement and recording of CBT, namely a temperature loggers (Mitchell *et al*., 2011; 2008).

The change in body temperature measured by the microchip (ΔIM-chip) and the data logger (ΔCBT) was greater in birds exposed to heat stress (30^{o}C, 40% RH or 30^{o}C, 70% RH) at the end of 3HS and the step down phase than in step up and post heat stress phases. Hence, it can be inferred that ΔCBT and ΔIM-chip were reliably related to the change in environmental conditions. A period of 3h exposure of broilers to 30^{o}C, 40% RH or 30^{o}C, 70% RH resulted in a moderate heat stress (MHS) causing a ΔCBT and ΔIM-chip of 0.7^{o}C and 0.5^{o}C, respectively, without mortality arising from hyperthermia. Broiler chickens with ΔCBT of 0.4-1^{o}C have previously been classified as experiencing MHS (Mitchell and Kettlewell, 1998).

In the current study, no relationship could be established between CBT and IM-chip. This could be attributed to the high baseline variation in muscle temperature between birds, despite effort made to position the microchip at the same depth in the breast muscle of each bird. Temperature recorded from the microchip showed a large variation, even during the pre-heat stress phase when all birds were kept in the same thermoneutral conditions (20^{o}C, 40% RH). The use of mean CBT data from the data logger for a period of 15 minutes may have smoothed temperature fluctuations compared with the instantaneous reading of the microchip, although greater stability in deep body temperature than in muscle would be expected.

Previous experiments using goats showed that microchips implanted in the retro-peritoneum gave the best agreement with CBT from a logger in the body-cavity whereas microchips implanted in the groin, muscle, flank and shoulder regions had a low agreement with CBT from the logger because there was a high variability between goats and between the different conditions in which the animals were tested (Torrao *et al*., 2011). In the current study, the
microchip was implanted in only one site (breast muscle) for two reasons. Firstly, the small body size of broilers limits the potential implantation sites compared to larger animals where several microchips can be implanted (Torrao *et al*., 2011; Lohse *et al*., 2010). Secondly, the breast muscle was selected to overcome the problems of microchip migration reported in other studies when chips were implanted subcutaneously (Chen and White, 2006).

At the end of the current study, all the microchips were recovered from the point of implantation. This implies that intramuscular implantation of microchips could help overcome problem of migration, although this study lasted for a relatively short period (15 days).

After standardization of temperature values, it was found that ΔIM-chip had a reliable relationship with ΔCBT, as confirmed by a Bland-Altman plot, and so could be used to predict the ΔCBT. In addition, the Bland–Altman plot showed that mean ΔIM-chip was only 0.1^{o}C less than ΔCBT, which confirms that the relationship is a sound one given that the threshold for the limit of agreement is 1^{o}C (Figure 5).

These promising findings demonstrate that microchips can be used to monitor ΔCBT of broiler chickens. This is particularly useful since ΔCBT is a parameter widely used as a clinical sign of the immediate condition of an animal (Kort *et al.*, 1998), or to classify the severity of heat stress as moderate or severe in broilers (Mitchell and Kettlewell, 1998) and layers (Wolfenson *et al.*, 1981). Indeed, a ΔCBT of 4^{o}C is lethal to birds (DEFRA, 2005). Therefore, ΔCBT estimated from a microchip could indicate the degree of heat stress to which the bird is exposed, and serve as a cue for the provision of alleviation measures to avert CBT from reaching a lethal point.

Infrared thermometers also showed promise for non-invasive monitoring of bird well-being. This study showed that 51% of the variation in CBT could be explained by WT. Moreover, ΔWT explained 75% or 67 % of the variation of ΔIM-chip or ΔCBT, respectively. This relationship between under wing and CBT may be due to peripheral blood flow arising from vasodilation of blood vessels when an animal is kept in a hot environment (Al-Tamimi, 2007). However, the use of under WT as an indicator of imminent hyperthermia may be limited because birds need to be restrained before temperature under the wing can be measured.

6. Conclusion

Although limited in the accuracy of absolute CBT, the ΔCBT of broiler chickens during exposure to MHS was reliably predicted from an intramuscularly implanted microchip, which is a less invasive procedure than the surgical implantation of a data logger. The use of a microchip in estimating ΔCBT implies that a baseline value of CBT must be scanned first. On the other hand, the under WT measured by an infrared thermometer gave a better prediction of the absolute CBT than IM-chip. It is suggested that these approaches may be applied in a range of experimental studies and for the routine assessment of health and welfare of birds maintained in experimental facilities and/or experimental farms. Therefore these methods potentially offer a major refinement over existing techniques.

7. **Acknowledgements**

The authors thank the technical staff of Newcastle University for their assistance during this experiment and the Veterinarians for their expertise in implantation of the temperature recording equipment. We thank Dr. Matt Leach for helpful comments on an earlier version of this manuscript.

References

Al-Tamimi HJ (2007) Responses of simultaneously recorded intraperitoneal and subcutaneous temperatures of black Bedouin goats to transient thyrosuppression during cold stress. *Livest. Sci.* **106**, 254-260. DOI:http://dx.doi.org/10.1016/j.livsci.2006.08.009

Blainey CG (1974) Site selection in taking body temperature. *Am J Nurs*. **74**, 1859-1861

Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurements. *The Lancet* **8**; 1 (8476): 307-310. DOI:10.1016/S0140-6736(86)90837-8

Borges SA, Da Silva AVF, Maiorka A (2007) Acid-base balance in broilers. *World's Poult Sci J* **63**, 73-81. DOI: http://dx.doi.org/10.1017/S0043933907001286

Brengelmann GL (1987) Dilemma of body temperature measurement. In Man in stressful environments- Thermal and Work Physiology. Eds. K. Shiraki, M.K. Yousef. Charles, C. Thomas. pp 5-22

Chen PH, White CE (2006) Comparison of rectal, microchip transponder, and

infrared thermometry techniques for obtaining body temperature in the laboratory rabbit (*Oryctolagus cuniculus*). *J Am Assoc Lab Anim Sci.* **45**, 57-63

Dallmann R, Steinlechner S, von Hörsten S, Karl T (2006) Stress-induced hyperthermia in the rat: comparison of classical and novel recording methods. *Lab Anim* **40**:186-193. DOI: 10.1258/002367706776319015

Daniels T (2009) The digestive system of a chicken. Accessed from https://poultrykeeper.com/digestive-system-problems/digestive-system-chicken/ on 10th October, 2016

Dawson WR, Whittow GC (2000) Regulation of body temperature, in Whittow, G.C. (ed.) *Sturkie's Avian Physiology*. San Diego: Academic press, pp. 343-390.

De Basilio V, Requena F, Leon A, Vilarino M, Picard M (2003) Early age thermal conditioning immediately reduces body temperature of broiler chicks in a tropical environment. *Poult Sci.* **82**, 1235-41

DEFRA 2005. Heat stress in Poultry- solving the problem. Department for Environment, Food and Rural Affairs 20p.

Flecknell PA, Waterman-Pearson A (2000) Pain Management in Animals. London: W.B. Saunders, p 184

Gerken M, Afnan R, Dorl J (2006) Adaptive behaviour in chickens in relation to thermoregulation. *Arch Geflügelk* **70**, 199-207

Jensen P, Toates FM (1997) Stress as a state of motivational systems. *Appl Anim Behav Sci.* **53**, 145-156. DOI:10.1016/S0168-1591(96)01156-2

Kort WJ, Hekking-Weijma JM, Tenkate MT, Sorm V, VanStrik R (1998) A microchip implant system as a method to determine body temperature of terminally ill rats and mice. *Lab Anim* **32,** 260-269

Lacey B, Hamrita TK, Lacy, MP, Van Wicklen GL, Czarick M (2000) Monitoring deep body temperature responses of broilers using biotelemetry. *J Appl Poult Res.* **9**, 6-12. DOI: 10.1093/japr/9.1.6

Lohse L, Uttenthal A, Enoe C, Nielsen J (2010) A study on the applicability of implantable microchip transponders for body temperature measurements in pigs. *Acta Veterinaria Scandinavica* **52**, 1-9. DOI: 10.1186/1751-0147-52-29

Medina LV, Coenen J, Kastello MD (2015) Global 3Rs – Making progress and

gaining momentum. *J Am Assoc Lab Anim Sci.* **54**, 115-118

Mitchell MA, Kettlewell PJ (1998) Physiological stress and welfare of broiler chickens in transit: Solutions Not Problems! *Poult Sci.* **77**, 1803-1814

Mitchell MA, Kettlewell PJ, Lowe J, Hunter RR, King T, Ritchie M, Bracken J (2011) Remote physiological monitoring of livestock - An implantable radio-telemetry system. In Proceedings of the 6th International Livestock Environment Symposium, Louisville, Kentucky, U.S.A., 21st-23rd May 2001. edited by Stowell, R. R., pp 535-541.

Mitchell MA, Kettlewell PJ, Villarroel-Robinson M, Harper E (2008) Systems for remote physiological monitoring in livestock: Assessing stress during transportation. In Proceedings of the International Conference on Agricultural Engineering & Industry Exhibition (AgEng 2008), Hersonissos, Crete – Greece, European Society of Agricultural Engineers, Agricultural and Biosystems Engineering for a Sustainable World, 23rd-25th June 2008. pp. OP-140.

Mrozek M, Fisher R, Trendelenburg M, Zillmann U (1995) Microchip implant system used for animal identification in laboratory rabbits, guinea pigs, woodchucks and amphibians. *Lab Anim.* **29**, 339-344.

Myles PS, Cui J (2007) Using the Bland-Altman method to measure agreement with repeated measures. *Br J Anaesth* **99,** 309-311. DOI: 10.1093/bja/aem214

Nicol CJ, Scott GB (1990) Pre-slaughter handling and transport of broiler chickens. *Appl Anim Behav Sci.* **28**, 57–73. DOI:10.1016/0168-1591(90)90046-G

Ojano-Dirain CP, Waldroup PW (2002) Protein and amino acid needs of broilers in warm weather: A Review. *Inter J Poult Sci.* **1**, 40-46

Quimby JM, Olea-Popelka F, Lappin MR (2009) Comparison of digital rectal and microchip transponder thermometry in cats. *J Am Assoc Lab Anim Sci.* **48**, 402-404

Rextroat J, Benish K, Fraden J (1999) Clinical accuracy of Vet-Temp instant ear thermometer. San Diego (CA): Advanced Monitors. 4p.

Robinson PJ (2001) On the definition of heat wave. *J Appl Meteor* **40**, 762-775. DOI: http://dx.doi.org/10.1175/1520-0450(2001)040<0762:OTDOAH>2.0.CO;2

Schwab RG, Schafer VF (1972) Avian thermoregulation and its significance in starling control. *Proceedings of the 5th Vertebrate Pest Conference,* 127-137.

Skuce PJ, Morgan ER, van Dijk J, Mitchell MA (2013) Animal health aspects of adaptation to climate change: beating the heat and parasites in a warming Europe. *Animal* **7**: s2: 333-345. DOI:10.1017/S175173111300075X

St-Pierre NR, Conbanov B, Schnitkey G (2003) Economic losses from heat stress by US Livestock industries. *J Diary Sci.* **86**, E52-E77. DOI: http://dx.doi.org/10.3168/jds.S0022- 0302(03)74040-5

Torrao NA, Hetem RS, Meyer LCR, Fick LG (2011) Assessment of the use of temperature-sensitive microchips to determine core body temperature in goats. *Vet Rec.* **168,** 328 – 334. http://dx.doi.org/10.1136/vr.c6200

Vale MM, Moura DJ, Naas IA, Pereira DF (2010) Characterization of heat waves affecting mortality rates of broilers between 29 days and market age. *Rev Bras Cienc Avic.,* 12, 279- 285. http://dx.doi.org/10.1590/S1516-635X2010000400010

Widowski T (2010) The physical environment and its effect on welfare. In Duncan, I. J. H. and Hawkins, P. (ed.) *The welfare of Domestic Fowl and other Captive Birds.* New York: Springer, pp 149-159

Wolfenson D, Frei YF, Snapier N, Berman A. (1981) Heat stress effect on capillary blood flow and its redistribution in the laying hen. *Pfluegrs Arch* **309**, 86-93

Yahav S, Shinder D, Tanny J, Cohen S (2005) Sensible heat loss: The broiler's paradox. *World's Poult Sci J* **61**, 419-434. DOI: http://dx.doi.org/10.1079/WPS200453

Table 1: Pearson correlation coefficient values (R) between core body temperature measured from a data logger (CBT) or an intramuscular temperature-ID chip (IM-chip) and surface body temperatures measured using an infrared thermometer at the end of 3 h of heat stress

	CBT	**IM-chip**	**Wing**	**Comb**	**Feet**	**Cloaca**
CBT	1.000					
IM-chip	0.358	1.000				
Wing	0.713*	0.190	1.000			
Comb	0.536	0.339	0.882**	1.000		
Feet	0.592	0.540	0.764*	0.802**	1.000	
Cloaca	0.192	0.619	0.342	0.331	0.537	1.000

*P<0.05, **P<0.001.

Table 2: Correlation coefficient values (R) between changes in core body temperature measured from a data logger (ΔCBT) or an intramuscular temperature-ID chip (ΔIM-chip) and four surface body temperatures measured from an infrared thermometer between the pre heat stress phase and at the end of 3 h of heat stress.

	ΔCBT	**ΔIM-chip**	**ΔWing**	**ΔComb**	**ΔFeet**	**ΔCloaca**
Δ CBT	1.000					
Δ IM-chip	0.714*	1.000				
Δ Wing	0.865**	0.819**	1.000			
Δ Comb	0.308	0.411	0.318	1.000		
Δ Feet	0.756*	0.416	0.672*	0.359	1.000	
Δ Cloaca	0.168	0.205	0.071	0.480	0.553	1.000

*P<0.05, **P<0.001

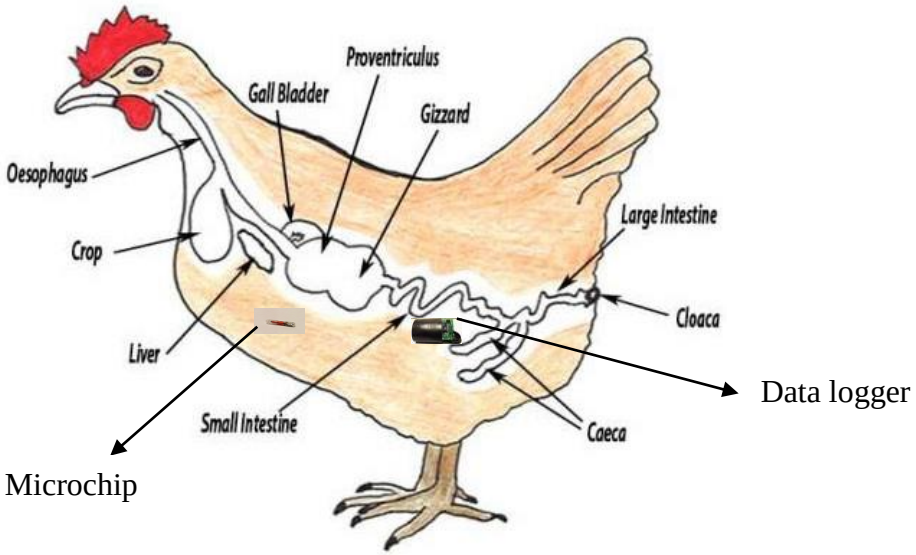

Figure 1: Diagram showing the specific locations of the data logger and microchip within the body cavity of a chicken. Source: Daniels (2009)

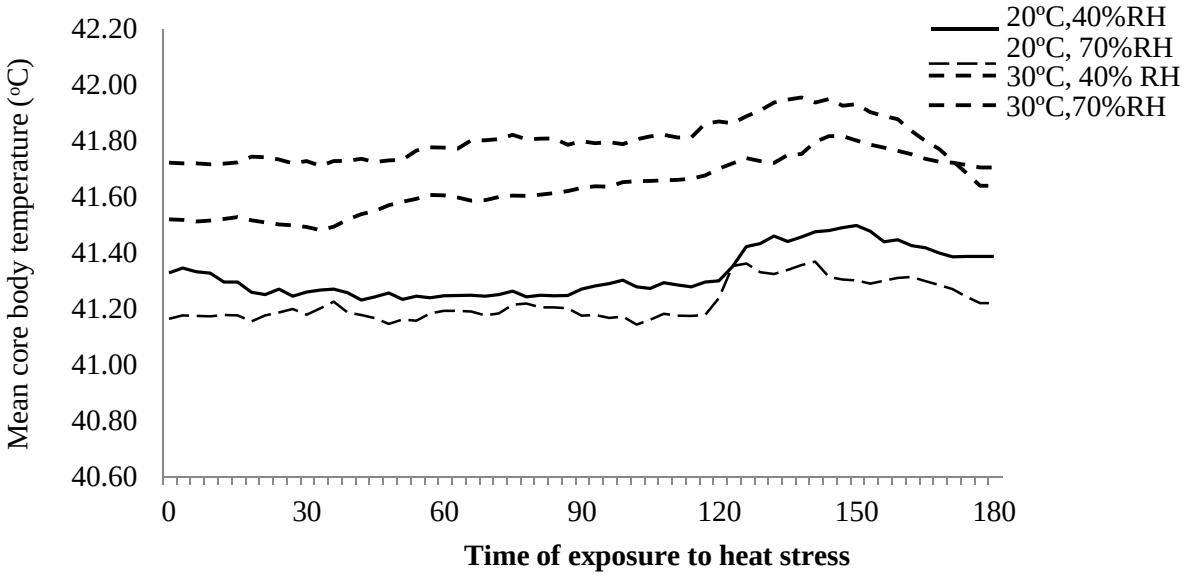

Figure 2: Core body temperature measured by a data logger every 3 minutes for a 3 hour period in broilers exposed to different temperature/relative humidity combinations (n=10 birds). Values are mean for 3 days

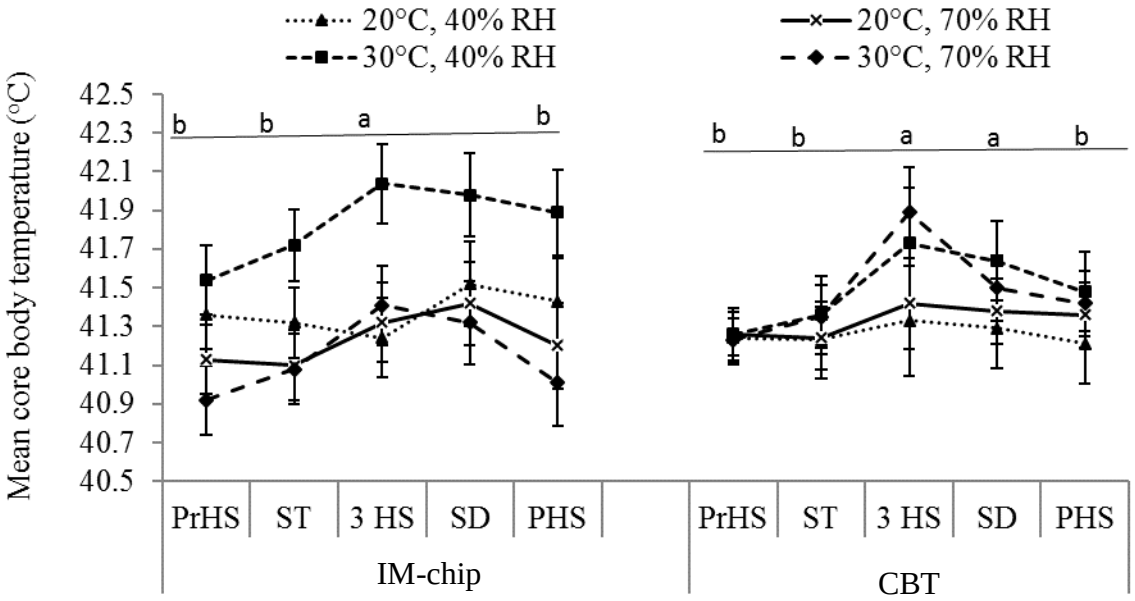

Figure 3: Core body temperature at different phases of the heat stress protocol measured by an intraperitoneal data logger (CBT; n=10 birds) and an intramuscular temperature-ID chip (IM-chip; n=12 birds). Values are mean for 2 days ±1 SEM. ab Means with different letters indicate the overall CBT value (single mean for all treatments during that phase) differs between phases ($P<0.05$).

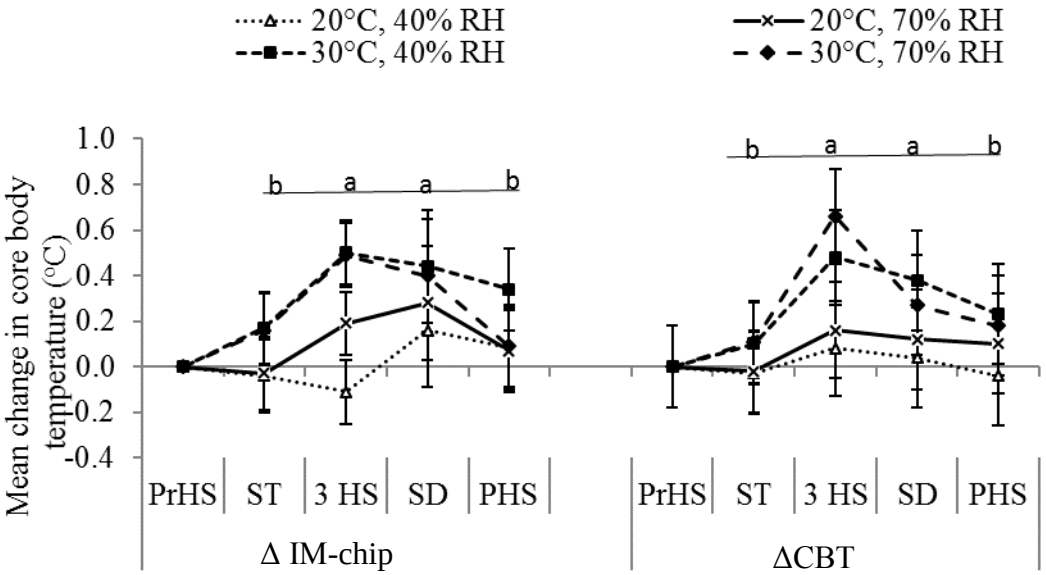

Figure 4: Change in core body temperature at different phases of the heat stress protocol of measured by an intraperitoneal data logger (CBT; n=10 birds) and an intramuscular temperature-ID chip (IM-chip; n=12 birds).Values are mean for 2 days ±1 SEM. ab Means with different letters indicate the overall ΔT value (single mean for all treatments during that phase) differs between phases ($P<0.05$)

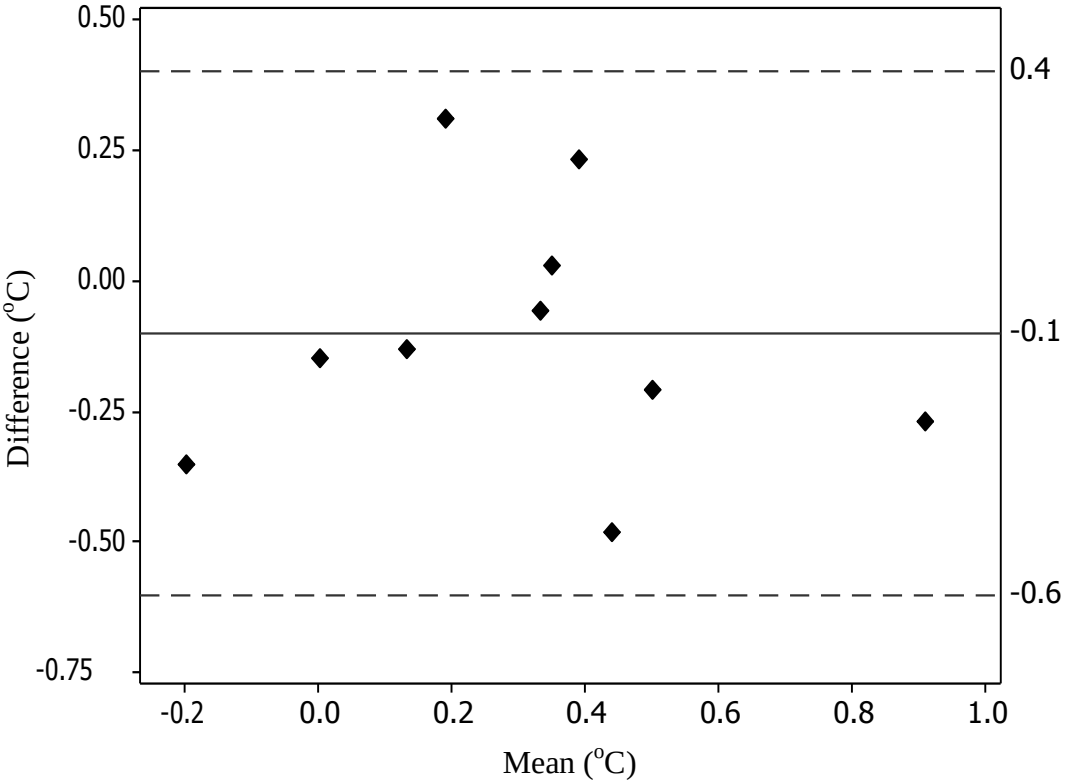

Figure 5: Bland-Altman plot showing the difference between the change in body temperature estimated by a data logger (ΔCBT) and that from an intramuscular temperature-ID chip (ΔIM-chip), n=10 at the end of 3HS. The difference in temperature estimation methods are plotted against the pairwise means. The solid line represents the mean difference between ΔIM-chip and ΔCBT. The distance between the broken lines represents the limit of agreement between the ΔIM-chip and ΔCBT. The Bland-Altman plot is a graph showing the difference between the ΔIM-chip and ΔCBT on the y-axis, and mean ΔIM-chip and ΔCBT on the x-axis.