HEAD EYE AND COMB TEMPERATURE CHANGES IN CHOOKS DURING HANDLING: The use of infrared thermal imaging in observing stress in chooks.

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Abstract

It is now widely accepted in the scientific community that animals suffer from both physical and emotional stress. Emotional stress has been linked to an increase in core body temperature and decrease in surface area temperature of at least 0.5 degrees Celsius in a wide number of species, a phenomenon known as Stress-Induced hyperthermia (SIH) (Edgar et al, 2013). Catching and handling are relevant events in an animals life. Wild prey animal's experience catching and handling by predators while animals raised for productivity experience catching and handling by Humans. Handling has been linked to the onset of stress-induced hyperthermia in many species, and Edgar et al (2013) provided evidence that handling causes a significant decrease in Head, Eye and Comb temperatures in Hens.

The aim of this current research was to replicate that of Edgar et al (2013). This research aims to use infrared thermography to measure changes in Chooks Head, Eye and Comb prior to, during and after capture and handling. It is the hope that this research will provide further evidence that handling causes stress-induced hyperthermia in Hens. Infrared thermal imaging is a non-invasive measure of surface temperatures. It is the hope that this research will provide evidence that infrared thermography is an effective non-invasive measure useful in animal welfare research.

13 Hens and 6 Roosters Head, Eye and Comb temperatures were measured using infrared thermal imaging during 20 minute pre measurement, handling (capture by a researcher and held for 5 seconds) and 20 minute post measurement periods. Average surface area temperatures for each of the 19 subjects were obtained every minute during pre-measurement, handling and post-measurement. Average temperatures were plotted and analysed to investigate any patterns of change.

Subjects Head, Eye and Comb temperatures experienced a significant decrease (more than 2 degrees celsius) from pre-measurement to handling. For Hens the Comb saw the most significant temperature drop during handling which is consistent with previous research. The Head saw the most significant decrease in temperature during handling for Roosters. Roosters Head Eye and Comb temperatures in post measurement went on to exceed those obtained in pre measurement while for Female subjects Head, Eye and Comb temperatures never returned to pre-measurement levels during the 20 minute post measurement.

Infrared thermography proved a useful and accurate measure of Chooks surface area temperature. A surface area temperature decrease of more than 2 degrees celsius was observed in all subjects. All subjects met the requirements for stress-induced hyperthermia after handling. Stress-induced hyperthermia had a significant and lengthy impact on Chooks, in particular Hens.

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Table of Contents

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List of Figures

List of tables

Table 9. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for the 13 female subjects (Hens) and the Average Head temperature taken during handling for the 13 female subjects (Hens).

Table 10. Shows the results of a paired samples T Test between the average Comb temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Comb temperature taken during handling for the 6 male subjects (Roosters).

Table 11. Shows the results of a paired samples T Test between the average Eye temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Eye temperature taken during handling for the 6 male subjects (Roosters).

Table 12. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Head temperature taken during handling for the 6 male subjects (Roosters).

Introduction

Civilisation and Animals

The relationships between humans and animals has become a popular area of research for Psychologists which is not surprising given that non-Human animals have been influential in the growth of human lifestyle and humanity. 8000 B.C saw the beginnings of human dependence on non-human animals for survival (History World International, 2007). The domestication and use of animals on a daily basis was the first step in the development of technology and science (Hodges, 1999). Animals have played a vital role in the evolution and survival of the human species in many ways including food production and farming, religion, medicine and transportation (History World International, 2007). Animals not only fill a 'need' in the lives of humans but also play a vital role in their comfort, attachment and life balance.

The use of animals in everyday life has allowed the production of food and quality of life to advance far beyond the primitive life style humans were once reliant on (History World International, 2007, Hodges, 1999). It was discovered around 12000 B.C that wolf pups could be tamed and used to track game, this led to the use of dogs in modern farming (History World International, 2007). 7000 B.C in the Middle East saw the taming of wild sheep, pigs and goats for wool, milk and lean meat which helped to keep humans healthier and warmer in the colder months of the year. Animal milk was a huge factor in the survival of many young infants and children when human milk was insufficient either in amount or nutrition (Hodges, 1999).

The domestication of animals saw the ability harness the power of animals and use it to enrich the soil and promote crop growth which led the way to pastoralism. Pastoralism became a popular means of farming and is still being widely utilised today to meet the growing demands of the expanding human population (History World International, 2007). Animal power led to the extraction of water from resources such as the ground and from nearby lakes and rivers for both irrigation to help with crop growth (Hodges, 1999). By integrating the use of animals into farming and food production many humans were freed up from daily labour. Only 5- 10% of the population was needed to farm (Hodges, 1999) leaving a growing work force outside of food production and advancements in other areas of human life such as science and technology (Hodges, 1999).

Animals are also used to create by-products such as art supplies; crayons, wax paper and paints, Rubber, candles, lipsticks, shaving creams and soaps along with wallets, furniture, clothes and shoes. The number of by-products created from animals make them a vital part of the economy. On average By-Products from a 1,000 pound steer make approximately 3.4 billion dollars a year in the United States (United States department of Agriculture, 2012).

Some archaeologist believe that animals such as horned cattle and bull were vital part of Humans mental health, being used as spiritual and religious symbols (History World International, 2007). Animals also became vital to humans physical health. Animal bones and horns were used to craft medical supplies such as needles (History World International, 2007).

Animals dramatically influenced the lives of Humans in the area of transportation. The domestication of smaller animals such as donkeys and horses in around 4000 B.C saw big changes in transportation for humans (Hodges, 1999). By using animals for transportation humans could travel further and faster with a heavier load. Animal transportation lead the way for increased trade of farm products and natural resources to people in other communities. Ridding animals continued to be the fastest way for humans to travel up until 170 years ago (Hodges, 1999).

The study of the relationships between humans and animals and the importance of them has become a popular area of interest (Beck & Katcher, 1996). If at any time the importance of animals is put into question one needs only to look at the relationship between a pet and its owner. Pets tend to become 'part of the family'. Dogs are quipped as being man's best friend'. Pets often have their own 'houses' and even their own form of entertainment; cats have elaborate scratching posts, dogs have chew toys and tennis balls, mice have running wheels and fish have underwater castles to explore . It is common for pets to become a social support system especially for growing children and the elderly (Hart, 1995). For growing children pets can provide a means of teaching responsibility and kinship and for elderly they can be a means of comfort and companionship (Hart, 1995. Hunt et al, 1992). According to Cox (1993) Families with pets are more likely to solve arguments easily, have closer 'family bonds' and a greater alliance to family.

Beck and Katcher (1996) suggest a link between relationships with animal's and improved human health. Beck and Katcher (1996) describe a study in which it was found that people who owned a pet had lower blood pressure then those who had no pets. The use of animals in rehabilitation of those who suffer from mental health disorders seems to support Beck and Katcher (1996) theory. Animals are often used in an area of mental health treatment/rehabilitation called Sensory Modulation. Animals are used to reduce stress and anxiety and combat the effects of a 'manic' episode. Allen et al (1991) suggested having a pet or a 'companion animal' could reduce stress. They provide evidence that pets had a positive effect on their owners stress when performing a mathematical problem in the presence of a Dog but no such positive effect was found in the presence of a close friend (Allen et al, 1991).

Animals preform an important task in the continuation of human life through production and farming, medical advancements, transportation and emotional and social relationships. Without animals humans would not have achieved the levels of advancements in science and technology or mass food productions which we thrive on today. It is important to maintain the health of the animals around us in order to enjoy the many benefits animals provide.

Animal welfare

"The greatness of a nation and its moral progress can be judged by the way its animals are treated." With one sentence Mahatma Gandhi spoke to millions about the importance of animals and their treatment. It is important to obtain and sustain the health and welfare of animals. To better understand animal welfare and its importance there are certain basic questions that must be answered, how do we determine if the state of care and treatment of an animal meet the standards of welfare required? And perhaps the most important question what is it? What is animal welfare?

It would be virtually impossible to find a universally agreed upon definition of animal welfare (Keeling et al, 2011). According to the Oxford English dictionary (1973) welfare originally meant 'well-being, happiness' but this is not an easy state to determine for an animal. Boom and Johnson (1993) define welfare as "a characteristic of an animal, not something given to it" (p.75) meaning it is not something that we can give to an animal but something we should help each animal maintain.

Animal welfare is such a vast and complicated issue, it has become standard for animal welfare to be describe on a scale (Appleby et al. 2011). A state of an animal's welfare is described in levels, from poor to good, in order to better identify where the animals needs are. Arguably the best well rounded definition of animal welfare are 'the five freedoms' (Kneeling et al, 2011). The five freedoms are as follows, freedom from hunger and thirst, freedom from discomfort and stress, freedom from pain, injury and disease, freedom from fear and freedom to perform behaviours that are natural to the animal (Farm Animal Welfare Council, 2009).

Animal welfare is not defined as one single state, but, is divided into three different states, physical, mental and behavioural/natural. According to Keeling et al (2011) the physical state was developed from frequently asked questions about the production and health of animals. Questions such as 'is the animal performing and producing well' and 'is the animal healthy and able to reproduce' helped form the physical state of animal welfare (Keeling et al, 2011). McGlone (1993) takes the physical state of an animal as the only measure of animal welfare. McGlone (1993) suggests that the animal is in a poor welfare state when there is a disturbance of physiological systems to the point where the animal's reproduction or survival abilities are endangered. The Physical state of animal welfare covers three of the five freedoms mentioned above, freedom from pain injury and disease, freedom from hunger and thirst and to some extent freedom to perform behaviours that are natural but it does not cover all five.

It is common for farmers and Veterinarians to hold a similar view to the one suggested by McGlone (1991). Farmers and Veterinarians tend the focus on physical health of the animal taking productivity, illness, disease and injury into consideration when it comes to animal welfare (Keeling et al, 2011) as deficits in these areas would impact greatly on their daily lives. Common physiological measures used in the physical aspect of animal welfare inclde enzymes, metabolites, age, breed, blood pressure and blood work (World animal net, 2009). The physical health of any animal is vital to its welfare (Cockram & Hughes, 2011). Disease and illness can negatively impact an animal's ability to stay alive and the quality of that life. Physical ailments can cause an animal pain and suffering, fear, distress, weakness, an inability to compete for resources and decrease their ability to avoid attacks (Cockram & Hughes, 2011). Although Physical health is important to any animals wellbeing it is not the only state of welfare involved in achieving overall animal welfare.

In earlier periods of animal research there was no scientific way to measure or prove that animals could feel therefore this notion was dismissed (Keeling et al, 2011). The scientific communities view on an animals ability to feel was forever changed in 1976 due to Donald Griffin and the publication of his book 'The Question of Animal Awareness'.

'The question of animal awareness' (Griffin, 1976) demonstrated a link between language, thought and experience. Griffin provided evidence of animal languages. Griffin (1976) reasoned, if animals can communicate, they could think and if animals could think, they can experience and experience requires feeling. If Animals could experience, human experience was not the only kind of experience and human feeling was not the only kind of feeling (Griffin, 1976).

It has since been established that animals can feel, feel both pleasure and pain and have subjective experience (Keeling et al. 2011). Animal welfare took a turn away from the physical health of animals and instead began to focus on measuring the emotional heath of animals (Duncan, 1996). Duncan (1996) went as far as to state that the emotional health of an animal is the most important aspect of animal welfare, if the animal feels unhappy, stressed or afraid then there is no welfare for that animal.

'Is the animal suffering?', 'is the animal stressed or in distress?', 'is the animal happy?' are all questions that relate to the mental welfare of animals. The Mental state of animal welfare covers two of the five freedoms 'freedom from discomfort and stress' and 'freedom from fear'. Members of the public and the media tend to be more focused on the animals mental state when discussing animal welfare, they want to know if the animals are happy and comfortable (Keeling et al, 2011). When addressing the mental welfare of an animal one requires knowledge of the animal, of its typical behaviours, one must observe behavioural traits that suggest changes in an animal's mental state (World animal net, 2009). Blood pressure measures and body temperature measures are also a useful measure in an animals mental welfare as both have been linked to stress in animals (Keeling et al, 2011).

Many have pointed to the 'unnaturalness' of animal housing as a leading cause for public involvement in animal welfare (Keeling et al. 2011). Some of the earliest research into animal welfare was conducted around how being indoors and in close housing proximity impacts on an animal's natural instincts and behaviour (Keeling et al, 2011). The small size of housing pens in considering the large number of inhabitants is a major concern of the public in terms of animal welfare. Being in constant close proximity to so many animals as well as the unnatural amount of human contact and husbandry procedures could be damaging to the animals mental health. Although obtaining information about the mental state of an animal is vital to the animals welfare it is not the only aspect that is important, it only covers two of the five freedoms of animal welfare.

'Can the animal perform natural behaviours?' 'Is the animal living a reasonably natural life?' 'Does the animal have habitat similar to what it would naturally gravitate towards?' all common questions surrounding the behavioural/natural state of animal welfare (Keeling et al, 2011). The natural state of animal welfare covers two of the five freedoms 'freedom from hunger and thirst' and 'freedom to perform behaviours that are natural to the animal'.

Vegetarians and vegans tend to protest the housing situations of animals and how natural the environment is more than any other area of animal welfare (Keeling et al, 2011). Although some restrictions on natural environments and behaviours are publically accepted such as zoos and keeping pets, the problem becomes in determining what restrictions are acceptable and what restrictions negatively impact animal welfare (Keeling et al, 2011). What restrictions impact welfare has influenced a range of experiments and scientific discussions on 'behavioural need'. A behaviour that is internally motivated not triggered by environment is classified as a behavioural need (Keeling et al, 2011). Animal research today is focusing on answering the question of behavioural need which behaviours are needed to sustain an animals health and prolong life and which aren't?. Although the natural or behavioural aspect of animal welfare is important, alone it only covers two of the five freedoms and does not paint a full picture of animal welfare.

According to Appleby et al (2011) the reason there is growing concern and involvement in research into animal welfare is the close link it has to the welfare of humans. A large portion of food production is reliant on animals, unhealthy animals produce less and lower quality products. Unhealthy animals could lead to a shortage of food, clothing and income for Humans.

Emphasis on animal welfare is placed on different welfare states by different groups in society. Although it would be ideal to achieve and maintain all of the 5 freedoms for optimal animal welfare it is often not possible. Achieving one form of freedom often comes at the price of another (Keeling et al, 2011) for example, in farming, providing animals with an open space rather than cages will allow freedom to perform natural behaviours but it may also lead to attacks from other animals and could cause disease and the spread of illness, violating two of the five freedoms to achieve one. Allowing free access to food and water may allow freedom from thirst and hunger but can also lead to weight gain and therefore discomfort and pain, one freedom at the cost of another. Due to the improbability of achieving all five freedoms it is often suggested that a choice in which freedoms and areas of wellness are most preferred by the animal, but as there is no language in common between humans and animals. Preference assessments were developed to help humans determine what is more desirable for the animal.

Preference assessments

W. H Thorpe was the first to link preference studies and animal welfare recognising that animal health and wellbeing were connected to an animal's choice in areas such as food and flooring material (Mills & Marchant-Forde, 2010). The basic assumption with animal welfare and animal preference is based around the principle that the more a particular resource is desired or preferred by an animal the more likely it is that the animal's health and welfare will suffer when access to that resource is hard to obtain or denied (Mills & Marchant-Forde, 2010). Preference tests allow animals to have a say in what environment, events and resources are available to them which in turn can positively impact animal health and welfare (Mills and Marchant-Forde, 2010). Keeping an animal from something it prefers or continuously forcing it to endure situations or environments it deems undesirable can cause a massive amount of Stress. Stress itself can negatively impact an animal's mental and physical health.

Stress in Animals

It is now widely accepted in the scientific community that animals suffer from stress. Stress is becoming one of the main topics of interest in animal welfare research.

Stress can be a common occurrence for animals, especially those bred for mass production and consumption. Stress can often be harmful and negatively impact animal health and welfare (Moberg, 2000). Animals suffering from stress can suffer physical responses such as disease, lack of reproduction and poor growth and development.

It is important to clarify what is meant by 'stress' in terms of animal research. According to Moberg (2000) stress can be best described in three stages, stage one, recognition, where the animal perceives a threat to its safety, where a stressor is identified. Stage one is not harmful to the animal, in fact it can be considered 'good' in the sense that recognition of danger can save an animal from attack. Stage two is the biological response to the perceived threat or stressor (Moberg, 2000). Stage two is where the body responds to the perceived threat in a way that consists of any combination of the four biological responses, immune, behavioural, neuroendocrine system and the consequence of the biological response.

A behavioural response is an active response, such as removing oneself from the environment. A neuroendocrine response consists of releasing hormones that are related to different bodily functions such as reproduction and metabolism which can manifest as, increased heart rate, changes in body temperature, sweating and heaving breathing (Moberg, 2000). The final response is actually the consequences of the biological responses. The final stage is where stress can move from 'good' to 'bad' and cause long term negative effects on an animal's health and welfare.

It is important to note that not all stress is bad (Moberg, 2000). Some stress responses can in fact be considered 'good' or non-threatening (Moberg, 2000). Some forms of stress have a longer and more detrimental effect on animal health and welfare than others, this is the difference between acute and chronic stress (Dantzer et al., 1997). Acute stress or an acute stressor causes a shorter less detrimental stress reaction from an animal, acute stressors can include bad weather such as a storm, considerable temperature changes and introduction to a new environment or introduction of a new animal (Dantzer et al., 1997). Chronic stress often lasts longer than acute stress and causes more biological changes in an animal which make it more likely that the animal will suffer and develop side effects such as disease (Moberg, 2000). When conducting stress related research in animals a measure of stress needs to be determined.

Stress Induced Hyperthermia

When studying ‘stress’ and discomfort in animals the data collection process can be extremely difficult. Behavioural and physiological measures have been developed to help provide evidence and measures for stress in animals. ‘Stress’ in several species has presented as an increase in core body temperature (Edgar et al. 2013), Blow flow and corresponding body temperatures have become a common measure for stress in animals. Emotional Stress resulting in a core temperature increase of at least 0.5 degrees Celsius is a phenomenon called stress-induced hyperthermia (SIH) (Bouwknecht, 2007). Strong links between self-reported stress and SIH in humans have led researchers to see SIH as a useful measure of emotional stress in Animals as well (Edgar et al, 2013).

Stress-Induced hyperthermia is caused by a level of emotional stress that results in Peripheral vasoconstriction. Peripheral Vasoconstriction is the constriction of blood vessels in the body. As the blood vessels constrict, the blood flow decreases. This restricts the flow of blood from the heart. When the blood flow is restricted from the heart the core body retains blood, this causes an increase in core body temperature. The increase in the core body temperature often results in a decrease in the surface area temperatures such as the hands and feet in humans and Head, Comb and Eyes of Chickens (Edgar et al, 2013).

According to Raynaud's phenomenon this process can be caused by Stress (Solomon et al, 1964).) Bouwknecht (2007) states that emotional stress does not have to be caused by a physical act, emotional stress can be caused by fear of an event or exposure to an aversive stimuli. SIH is caused by emotional stress. An increase in core body temperature can be difficult to measure and can be invasive for animals. A decrease in surface area temperature of at least 0.5 degrees Celsius is easier to measure so is often used to identify stress-induced hyperthermia in animals (Edgar et al, 2013).

When measuring stress in animals, it would be counterproductive to use a stress inducing method. Effective non-invasive techniques are preferable but can be more difficult to come by and can be less accurate than invasive measures.

Infrared thermal imaging

A wide range of techniques and practices have been developed and utilised to measure and determine levels of stress in animals some are more invasive than others. Core body temperature of animals has customarily been obtained through the use of internally implanted data loggers (Edgar et al, 2013), this process is effective but invasive.

Recent developments in Thermal imaging technology has made it a viable and accessible means of obtaining readable body surface temperatures in a non-invasive way (Edgar et al, 2013). Thermal imaging cameras work by providing readable surface area temperatures by detecting infrared radiation from the animal's body surface area. Infrared thermal imaging cameras provide an effective non-invasive way to measure the effects of stress on an animal.

Handling

Edgar et al (2013) claim catching and handling are extremely relevant events that occur in any prey animal. Prey animals can associate catching and handling with pain and loss of life. For animals that have no real natural defence mechanism, such as Chickens, catching and handling can be particularly stressful. Handling is not only a relevant event for wild animals but is also relevant for animals in a commercial setting. The process of slaughter for commercial chickens begins with the capture and handling of the animal. Cabanac and Aizawa (2000) presented preliminary evidence that handling caused stress-induced hyperthermia in Hens, they found a decrease in surface area temperature after handling in two of the three Hens being monitored (Cabanac & Aizawa, 2000).

Findings were deemed preliminary due to the small number of participants and small number of temperature readings obtained (Edgar et al, 2013). More research into the effects of handling on Hens is needed.

Edgar et al. (2013) used infrared technology to measure hens head, eye and comb temperatures during handling to ascertain whether handling causes stress-induced hyperthermia in hens. Edgar et al. (2013) found that handling of hens had an effect on surface body temperature.

The aim of this research is to reproduce the above results. Using 13 female subjects, 6 male subjects and similar infrared thermal imaging technology the effects of handling on the blood flow and surface area temperatures of Chickens will be measured. In this research the hope is to promote the use of Infrared Thermal Imagining technology in animal welfare research and to provide evidence that Handling causes Stress-induced hyperthermia in both Hens and Roosters.

Based on the findings of Edgar et al (2013) it is expected that handling will cause stress-induced hyperthermia, a decrease in surface area temperatures in Hens by at least 0.5 degrees Celsius. It is expected that the Comb will be the most affected area during handling showing the biggest temperature decrease (Edgar et al, 2013) while

Head and Eye temperatures decrease from post measurement by a significant amount. It is expected that infrared thermal imaging will prove a useful, non-invasive measure of stress in the subjects (Edgar et al, 2013).

Method

Subjects

19 chooks (gallus gallus domesticus), 13 Hens and 6 Roosters (previously unused in research experiments) were obtained from an animal research lab in the Waikato region. The chooks were all between the ages of 9 – 11 months. The hens were all in good physical condition with no medical or physical conditions. Subjects were excluded from the experiment for one of two reasons, if they had a substantial bend in their comb which would make obtaining accurate temperature measures difficult or if they had previously been used in experiments and had therefore been over exposed to human handling. All subjects had limited exposure to handling and human contact prior to the experiment. *Figure 1.*

Figure 1. Picture of housing Aviary.

Housing

The subjects were moved from an outdoor pen and were placed in one of two indoor aviaries (in the same rom separated by mesh) according to their previous housing groups (to limit stress). The subjects were housed in 2 groups. Each group was placed in an indoor aviary measuring 2.5m x 3m x 2.5m high comprised of orange mesh, cardboard and tables for door access (*figure 1)*. The Aviary was bedded with 3cm of wood shavings which were cleaned and replenished daily. Subjects were given free access to wheat and water (3L of fresh water and wheat were placed in each aviary daily). A laying box (*Figure 2)* and a dustbathing box (*Figure 3)* were also placed in each aviary. A control switch was used to control the light schedule (*Figure 4)* which was maintained at 12L:12D.

Figure 2.

Figure 2. Picture of laying box used

Figure 3.

Figure 3. Picture of dustbathing box used

Figure 4

Figure 4. Picture of Light controller used

Identification

Group one was identified as the YELLOW group and consisted of 10 subjects, 3 Roosters and 7 Hens. Groups two was identified as the BLUE group and consisted of 9 subjects, 3 Roosters and 6 Hens. Each subject was weighed and received 2 colour identification bands. The first coloured band was the group colour, either Yellow or Blue. The second coloured band was to identify each subject as an individual (in place of a number system) examples include Blue Light Blue and Yellow Red to which the subjects will be referred to throughout the research process.

Procedure:

Acclimatization

Once subjects were tagged and placed in the aviary the next 3 days were acclimatization days, subjects had three days to acclimatise to their new environment with minimal human interference.

Table 1.

Days	1	2	3	4	5	6	7	8	9	10	11
Yellow group	ACL	ACL	ACL	HAB	REST	HAB	REST	HAND	REST	HAN	REST
Blue group	ACL	ACL	ACL	REST	HAB	REST	HAB	REST	HAN	REST	HAN

Table 1. Showing the experiment schedule for Blue and Yellow groups where ACL= Acclimatisation, HAB= habituation, HAN= Handling experimental day and REST is a rest day where subjects had minimal contact with researcher.

Habituation

Following the first 3 acclimatisation days, the 4th day was the beginning of the habituation phase. The habituation phase lasted 4 days, 2 days for each group (See *table 1)*. Each day in the habituation phase the researcher spent 4 hours inside one side of the housing pen with the thermal imaging camera (See *Figure 5* for schedule*)*. This process was used to expose the subjects to both the experimenter and the thermal imaging equipment that would be used in the experiment in an attempt to prevent them becoming extraneous variables and help limit their effect on stress readings.

Figure 5.

Figure 5. Picture of Thermal imaging Camera and laptop equipment used in research.

Order of testing

Day's 8-11 were testing days. 5 subjects were tested on days 8-10 and 4 were tested on day 11. Days 8 and 10 were used to Test YELLOW group subjects and days 9 and 11 were used to test BLUE group participants (See *Table 1* for testing schedule). Order of testing was randomly assigned. Testing Days consisted of a 20 Minute pre-measurement phase, A 5 second handling phase and a 20 minute post-measurement phase for each subject.

Pre-measurement

Pre-measurement took place in the subjects housing pen. An infrared thermal imaging camera (ThermaCam S60) was used to record subjects head regions (including Comb and Eye). The aim was to obtain a side on image of the subjects Head to get a clear picture of the Comb and Eye. The camera was held by the researcher and plugged into the laptop at all times. The researcher remained at an approximate distance of 1 metre from the subject during filming. Pre-measurement filming lasted 20 minutes for each subject with the objective of obtaining a useable image per minute of footage (20 reading for each the Head, Eye and Comb). The Thermal camera was set to an emissivity of 0.98. Temperature and humidity readings were taken at the beginning of every recording session. At the 20 minute mark the handling phase would take place.

Handling

Handling took place in the subjects housing pen. The condition began by an experimenter entering the housing pen and catching the subject by placing their hands over the hens wings and body. Once the experimenter had hold of the subject they would hold them side on approximately 1 m from the researcher and thermal imaging camera. The researcher would use the thermacam to record the subject side on for 5 seconds at which point the experimenter would place the subject back on the ground in the housing pen and leave the environment. The Thermal camera was set to an emissivity of 0.98. Temperature and humidity readings were taken at the beginning of every recording session.

Post-measurement

Post-measurement period also took place in the subjects housing pen. Post-measurement would take place after handing. Post-measurement was the same process as pre-measurement but took place after handling. Post-measurement lasted for 20 minutes for each subject. The Thermal camera was set to an emissivity of 0.98. Temperature and humidity readings were taken at the beginning of every recording session.

Thermal imaging Analysis

Once all pre-measurement, handling and post-measurement phases were complete for the 19 subjects the Data Analysis process began. The infrared thermal imaging videos were imported into the Thermocam Researcher PRO 2.7 SOP software. Each measurement phase for every subject resulted in a new recording session (approximately 3 recording sessions per subject). Due to technical difficulties there ended up being a total of 74 recording sessions between the 19 subjects.

Before a recording session was open object parameters were adjusted, emissivity was set to 0.98, reflected temperature, atmospheric temperature and humidity readings obtained during the recording session were inputted and distance was set to 1 m (this process occurred for every new recording session opened). Once a recording session was open Results and Histogram tabs were opened. Histogram settings were changed to read GENERAL 64 (classes) and the axes % was changed to AUTO. Histogram Axes were adjusted accordingly to accommodate each frame chosen for analysis.

Once a recording session was open, the researcher observed the footage and at approximately every minute interval the footage was frozen and a suitable frame was chosen for analysis. A suitable frame consisted of a side on, clear view of the subjects Head, Eye and Comb. Once a suitable frame was obtained a measurement region was emphasised by a tracing tool, a polygon was drawn around the entire head, Comb and around the Eye (See *Figure 6, 7, 8)*. Each frame provided a Head, Eye and Comb region to analyse. Once the measurement region was emphasised the total number of pixels in the designated area were obtained (in order for an area to be considered useable for measurement it had to return a number of at least 4.9 pixels).

Once it was confirmed that the measurement area had at least 4.9 pixels Histogram data (see *figure 9*) was exported to an excel spreadsheet. Histogram data contained average and maximum temperatures from the measurement area. Spreadsheet data was used for the data analysis.

Figure 6.

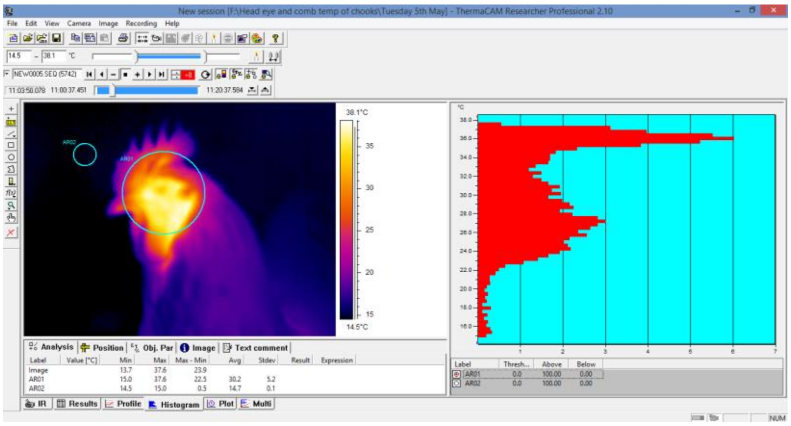


Figure 6. Infrared thermal image showing example of Head measurement region obtained from Thermacam researcher pro 2.7.

Figure 7.

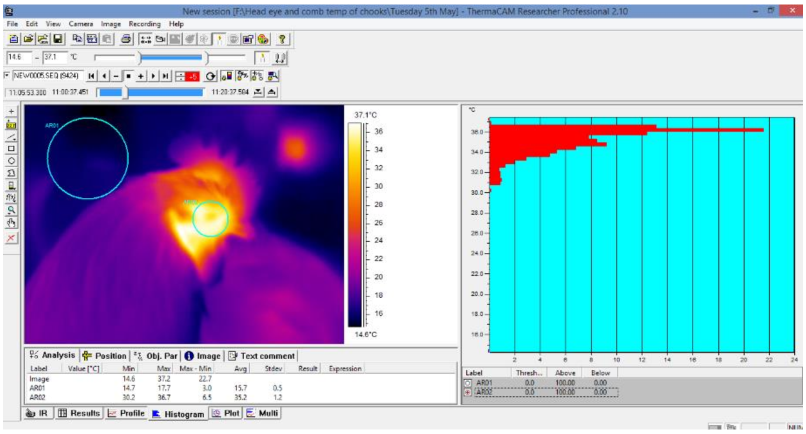


Figure 7. Infrared thermal image showing example of Eye measurement region obtained from Thermacam researcher pro 2.7.

Figure 8.

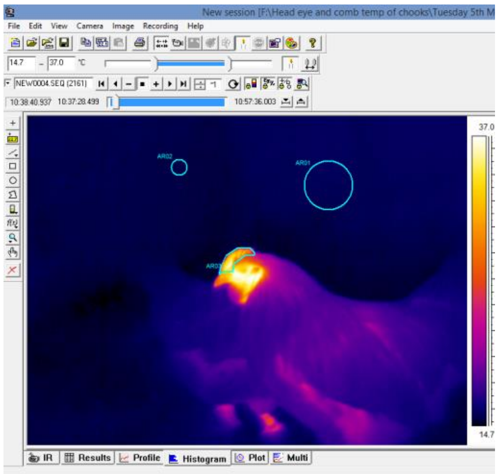


Figure 8. Infrared thermal image showing example of Comb measurement region obtained from Thermacam researcher pro 2.7.

Figure 9.

Date	ID	Condition	Body area	Time	Max	Avg	Total pix	no. of pixels
5/05/2015	YDG	pre 1	Head	10:16:34	37.3	27.4	1581	8.0631
	YDG	pre 1	eye	10:16:34	35.8	33.3	177	6.0003
	YDG	pre 1	comb	10:16:58	27.9	24.8	181	4.9775
	YDG	pre 2	head	10:17:26	37.3	29.2	1581	15.0195
	YDG	pre 2	eye	10:17:26	36.2	33.8	221	5.9891
	YDG	pre 2	comb	10:17:26	28	23.4	390	6.006
	YDG	pre 3	head	10:19:12	36.2	26.4	1073	5.0431
	YDG	pre 3	eye	10:19:12	35.3	32.4	221	11.0058
	YDG	pre 3	comb	10:19:12	26.3	23.8	240	7.992

Figure 9. Excel spreadsheet data columns. Showing data entered from histogram in Thermacam researcher pro 2.7.

Data Analysis

There were 3 individual data points that are unexplained. One temperature spike occurred in one male subject during pre-measurement and two temperature spikes occurred in another male subject also during pre-measurement (See *Figure 11).*As temperature spikes did not occur in the majority of subjects, were not consistent and only occurred in one body part, they were removed from the analysis. There is no way to accurately say what caused the three temperature spikes so have been put down to either a human or equipment error.

Once unexplained temperature spikes were removed from the data analysis data was analysed using PAWS Statistics 18. A series of paired sample T tests were carried out to determine any significant changes in temperature during testing. To determine the effect handling had on Hens Head, Eye and Comb temperature paired sample t tests were carried out between average Hen Head, Eye and Comb temperatures taken at pre measurement 20 minute and those taken during handling. The same was done for Roosters and again with all 19 subjects, to determine if handling had a more significant effect on male or female subjects. To determine the length of time the effects of handling lasted on the subjects, paired sample dependent T tests were carried out between average Head, Eye and Comb temperatures at the pre 20 minute mark and the post 20 minute mark for Hens subject group and Roosters and another with all 19 subjects to determine if there was a difference between recovery in Male and Female Chooks.

Results

Figure 10 (pages 32-36) is a series of line graphs showing the average temperature of the Head, Eye and Comb for 13 hens during a baseline, handling and post handling conditions. The X access has a data point for every minute over a 20 minute pre measurement period, a 5 second handling condition and a 20 minute post measurement period. The Y axis shows the average body part temperature for each bird in degrees Celsius. Each of the three plotted lines represent a body part of the hen, Green corresponding to Eye, Blue corresponding to Head and Red corresponding to Comb.

As was anticipated from the literature the most consistent trend was a drop in temperature during the handling phase. Three 2 sample dependant T tests of all 13 Hens comparing the average temperature of the Head, Eye or Comb in the 20 minute pre measurement condition to the corresponding body parts average temperature during handling returned significant results for the Head $t(12)=14.248$, $p<0.05$, Eye $t(12)=11.592$, $p<0.05$ and Comb $t(12)=6.990$, $p<0.05$. The dramatic decrease in the Head, Eye and Comb temperatures during handling could be an indication of Stress-induced hyperthermia (SIH).

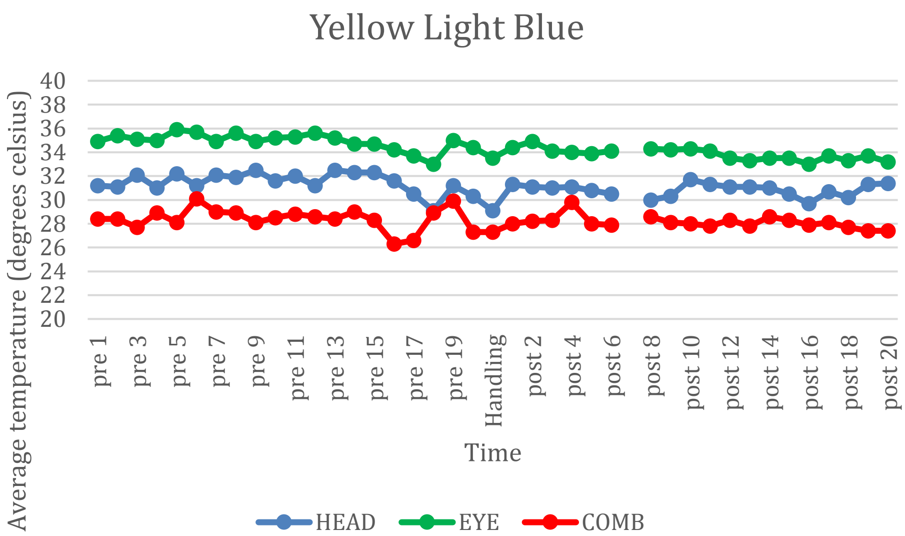
The second trend that can be observed is the relatively stable measures throughout the pre measurement and post measurement periods. There appears to be very little variability in the surface area body temperatures throughout these measurement periods (with the exception of subject Yellow Light Blue).

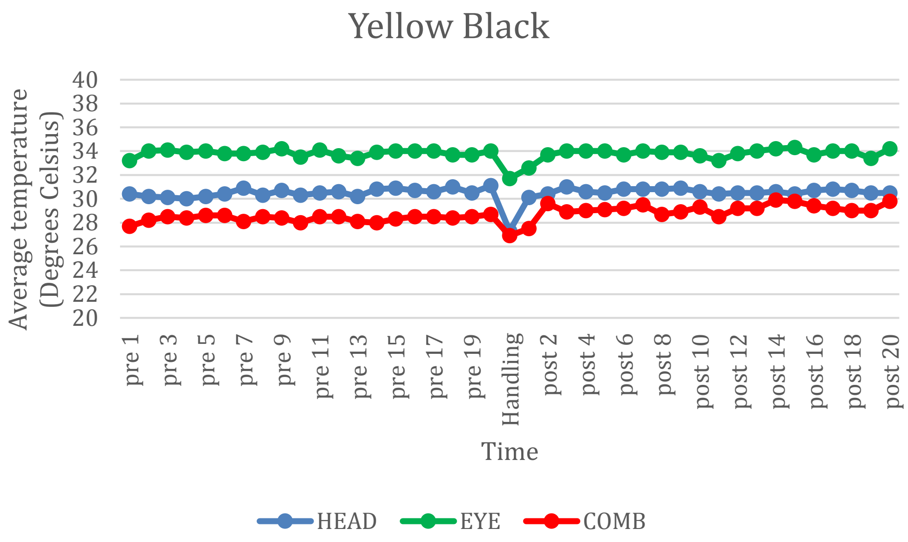
Figure 10 shows a reliable steady rise in the temperature of the Head, Eye and Comb across the majority of the subjects during the pre-measurement period (with the exception of Yellow Light Blue and Yellow Black). This trend could suggest habituation to the measurement process.

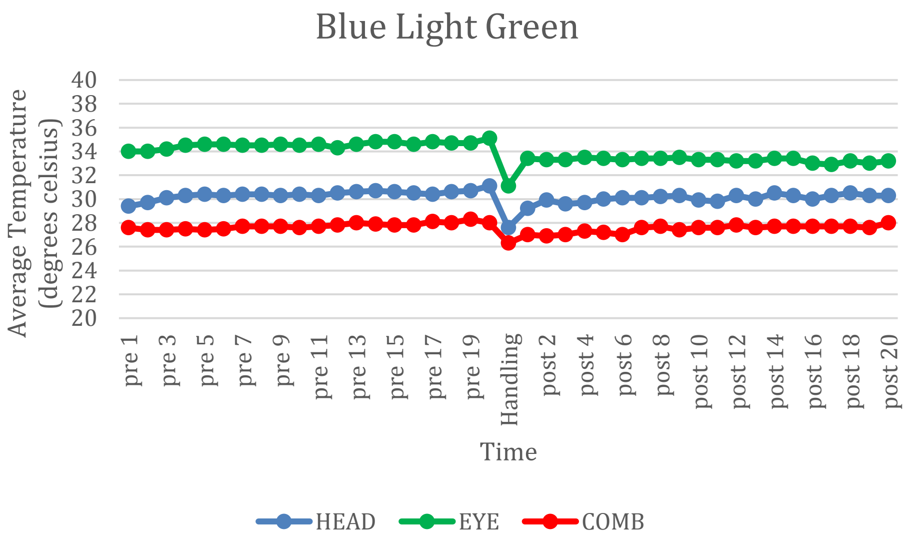
A steady rise in temperature can also be observed for the majority of the subjects throughout the post measurement period as time after handling increases.

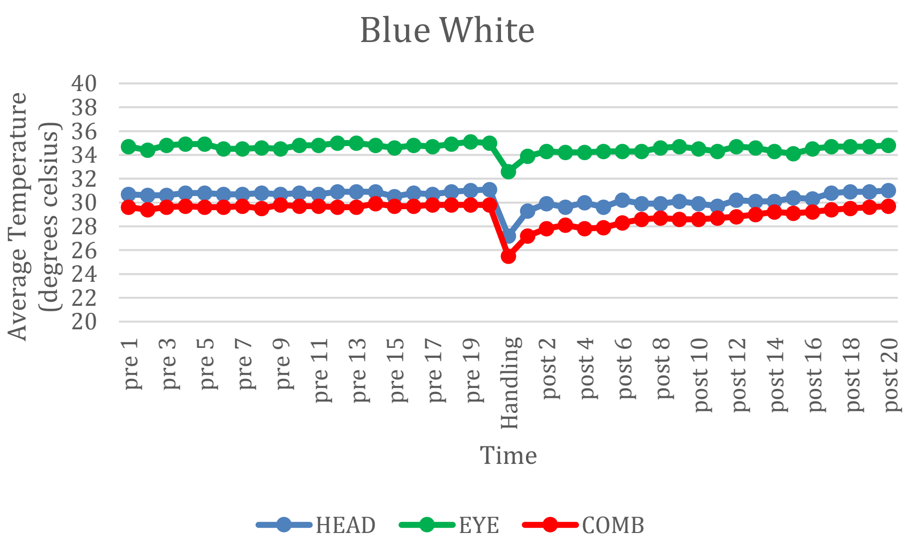
There is a clear distinction in the temperature of the majority of the subject's body parts. The Eye is consistently the hottest body part followed by the Head. The Comb is consistently the coldest. There is one exception to the temperature order, in subject Blue Dark Green, the Head and Comb appear equal during the Pre measurement period.

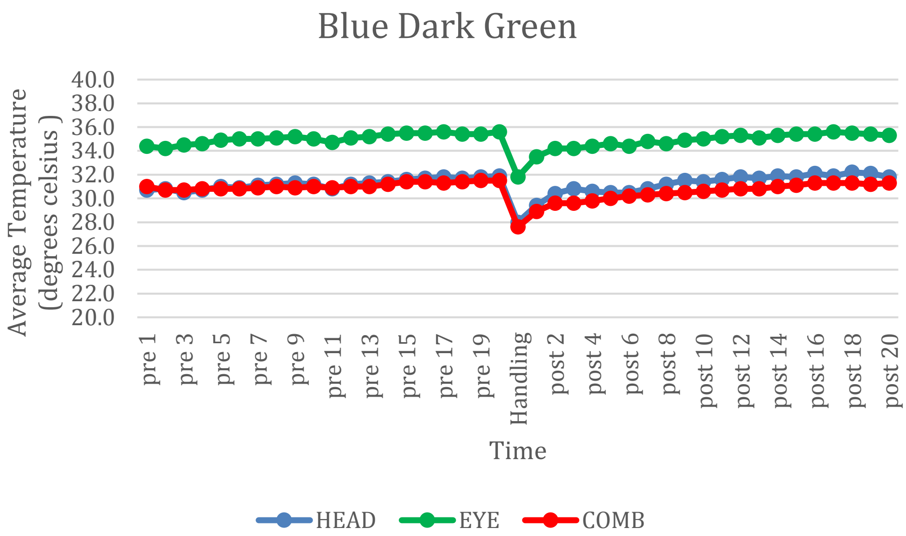
Figure 10.

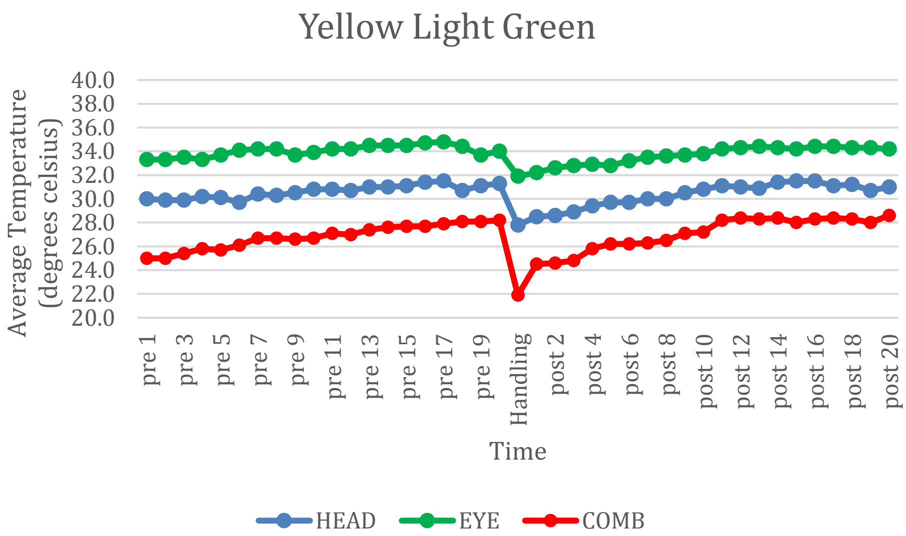


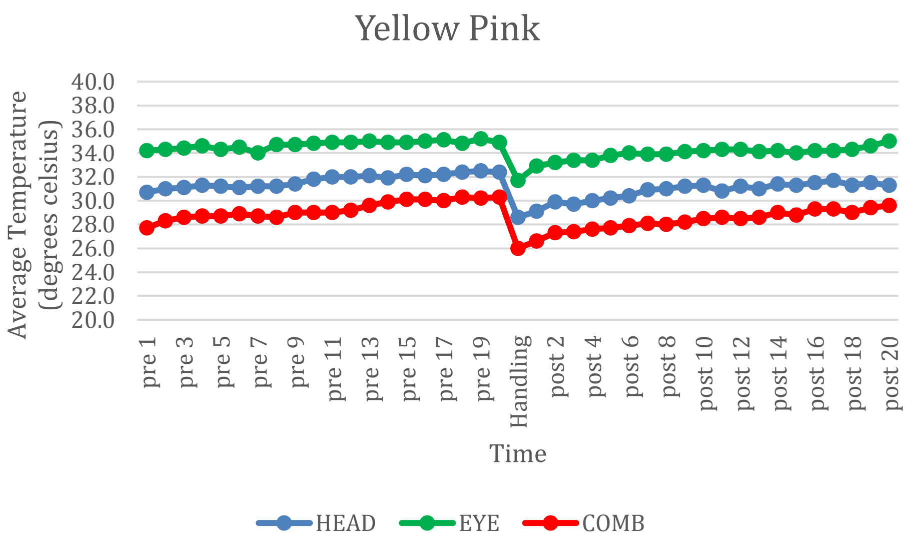

Yellow Black
Average temperature (Degrees Celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

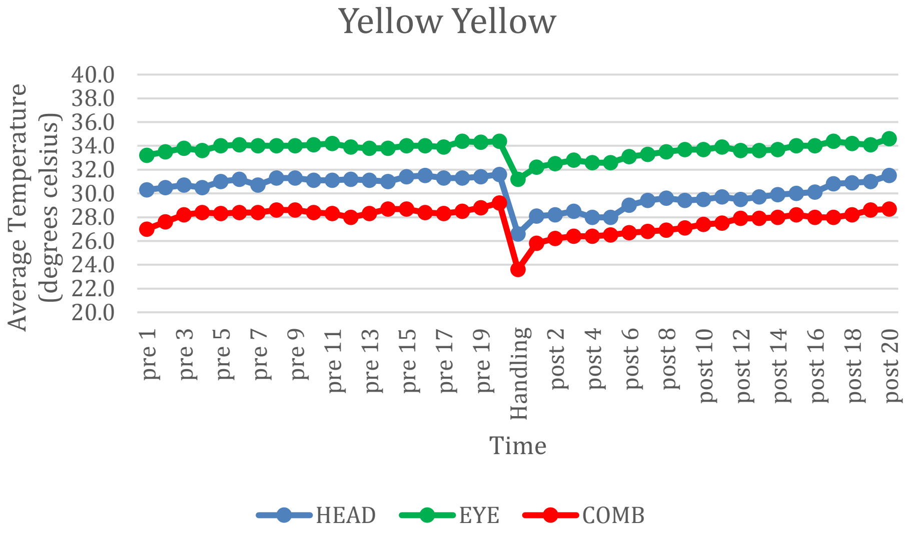

Blue Light Green
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

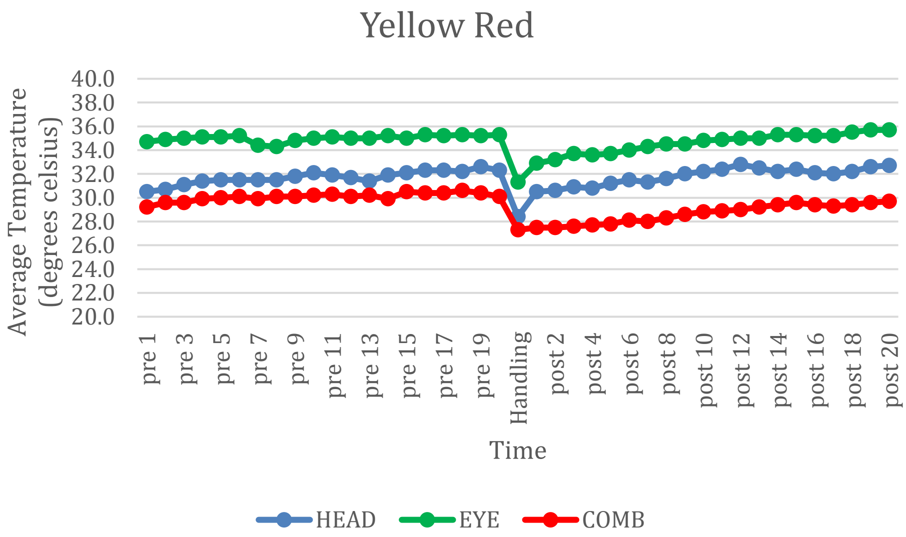

Blue White
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

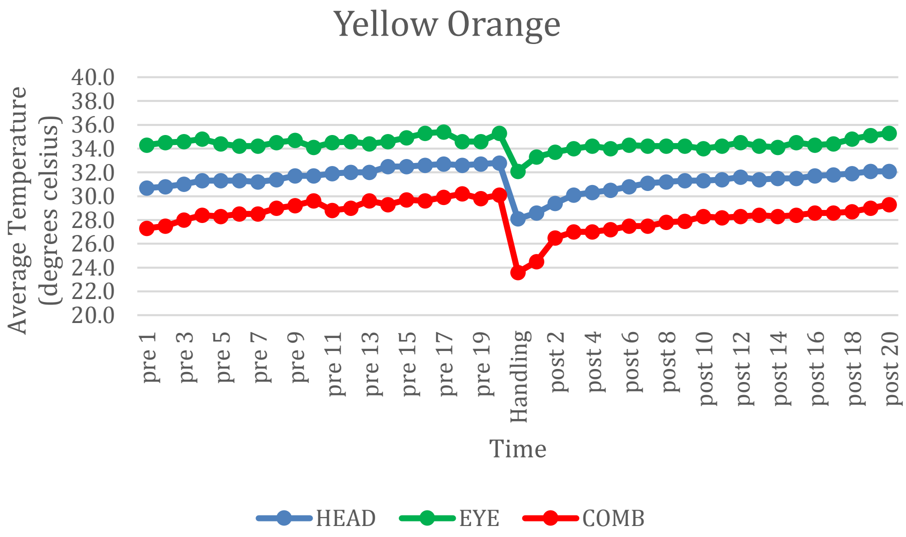

Blue Dark Green
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

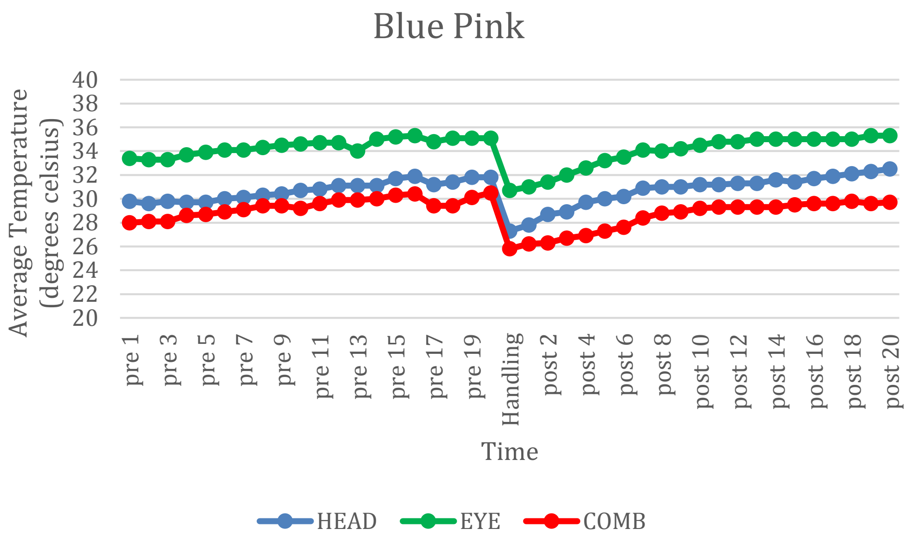

Yellow Light Green
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

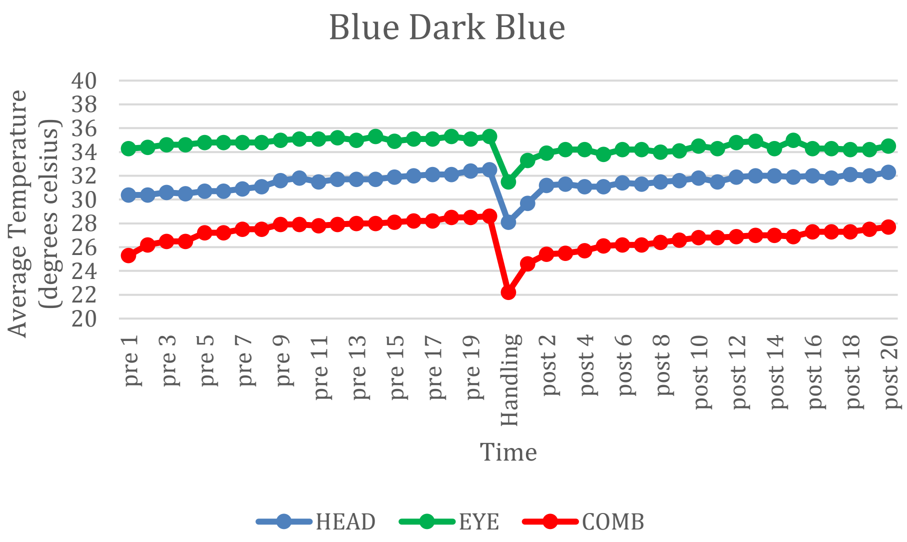

Yellow Pink
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Yellow Yellow
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Yellow Red
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Yellow Orange
Average Temperature (degrees celsius)
40.0
38.0
36.0
34.0
32.0
30.0
28.0
26.0
24.0
22.0
20.0
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Blue Pink
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Blue Dark Blue
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

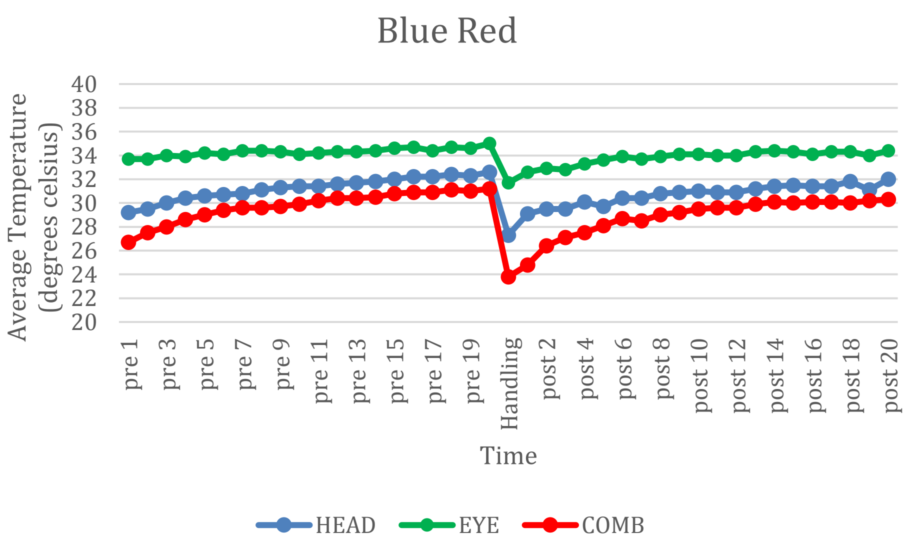

Blue Red
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

Figure 11 (page 38-40) is a series of line graphs showing the average temperature of the Head, Eye and Comb in 6 Roosters during a baseline, handling and post handling condition. The X and Y axes in *figure 11* represent the same as those in *figure 10.*

Figure 11 shows a consistent drop in the Head, Eye and Comb temperatures for the majority of subjects during the handling phase. Three 2 sample dependant T tests of all 6 Roosters comparing the average temperature of the Head, Eye or Comb in the 20 minute pre measurement condition to the corresponding average Head, Eye or Comb temperature during handling returned significant results for the Head $t(5)=5.260$, $p<0.05$ and Comb $t(5)=6.617$, $p<0.05$ however there was no significant difference found for the Eye $t(5)=2.231$, $p>0.05$.

Relatively stable measures can be observed throughout the pre measurement and post measurement periods in four of the six subjects in *Figure 11*. There appears to be very little variability in the temperatures of the Head, Eye and Comb throughout these measurement periods with the exception of subjects Yellow White and Yellow Dark Green.

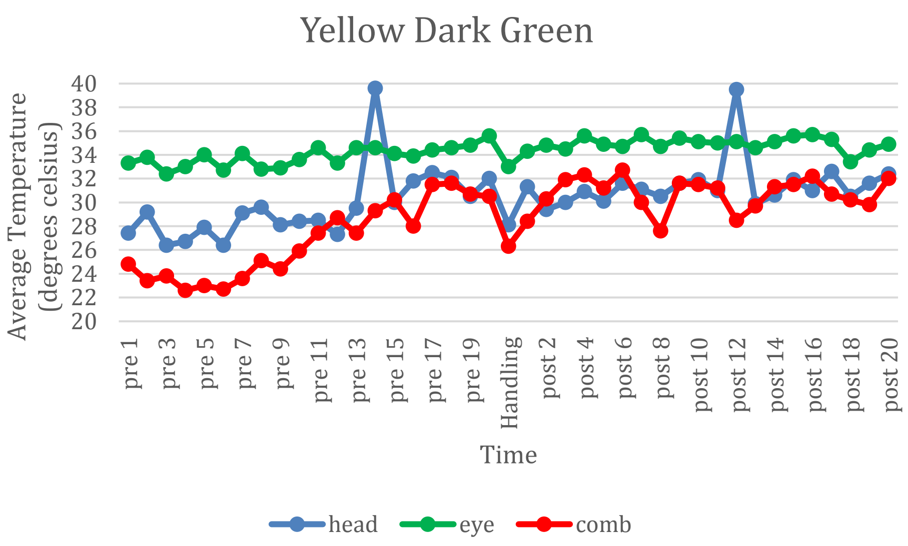
Figure 11 does not shows any reliable steady rise in subject's temperatures during the pre-measurement period.

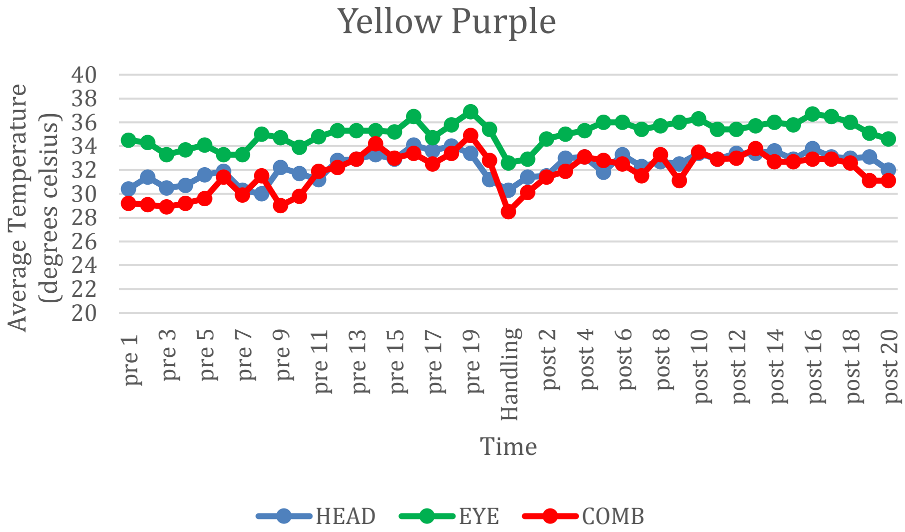
A steady rise in temperature can be observed in half of the subjects (Blue Purple, Blue Orange and Blue Light Blue) throughout the post measurement period as time after handling increases.

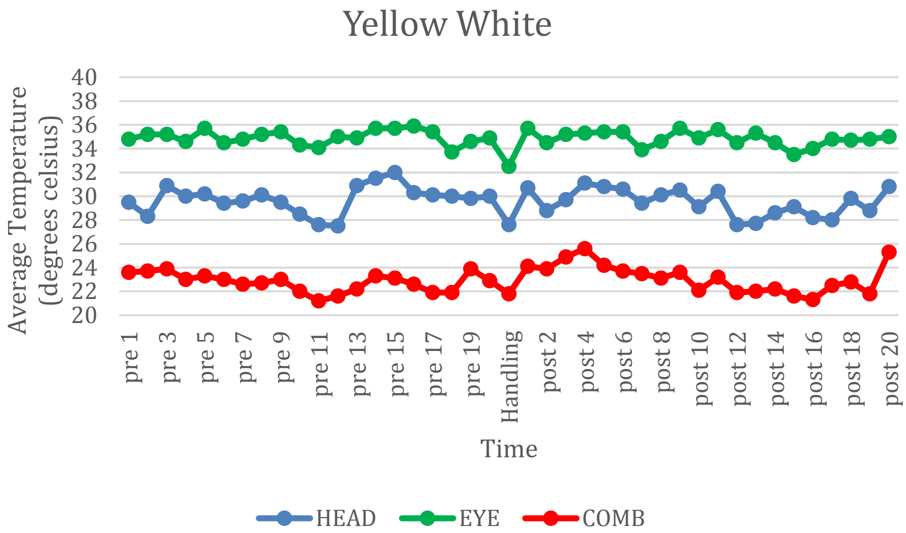
Figure 11 shows a clear distinction in the temperature of body parts in subjects Yellow White, Blue Orange and Blue Light Blue while Subjects Yellow

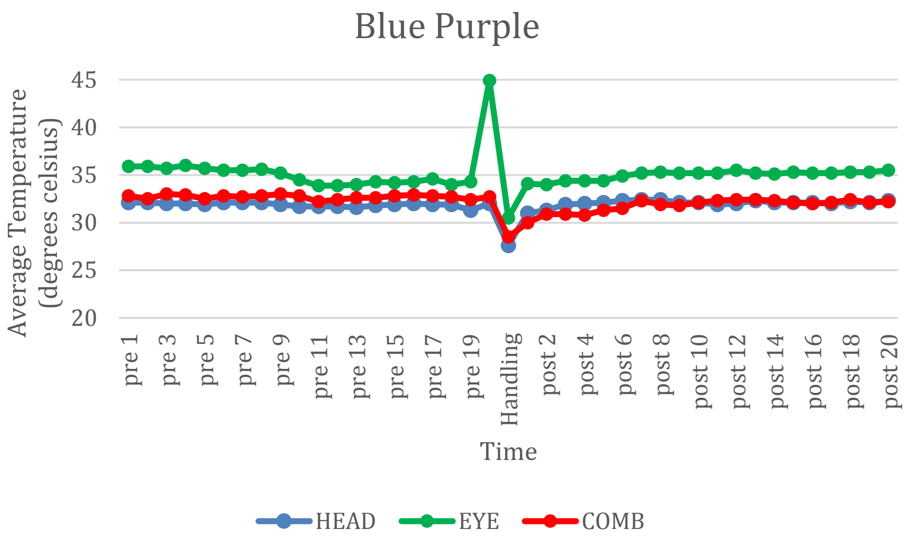
Dark Green, Yellow Purple and Blue Purple show some cross over. The Eye is consistently the hottest body part across all subjects and measurement periods. These findings are not consistent with previous research (Edgar et al, 2013). Previous research would suggests the Head was the hottest body part followed by the Comb (Edgar, et al, 2013) finding the Eye to have the coldest temperature. The Head and Comb show some cross over in temperature rankings with the Head being hotter consistently across measurement periods in 3 subjects but in the other three, the comb periodically exceeded the temperature of the Head.

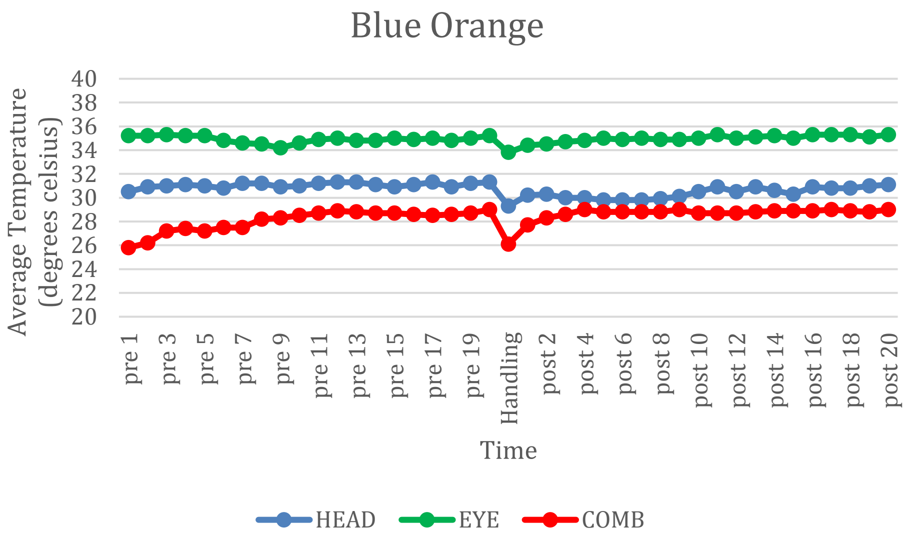
*Figure 11***.**




Yellow Purple
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Yellow White
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Blue Purple
Average Temperature (degrees celsius)
45
40
35
30
25
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB


Blue Orange
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

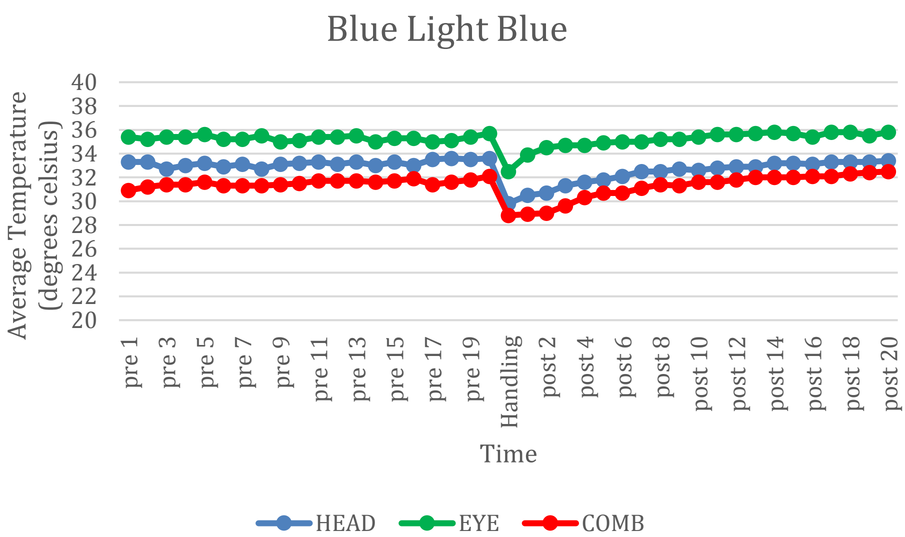

Blue Light Blue
Average Temperature (degrees celsius)
40
38
36
34
32
30
28
26
24
22
20
pre 1
pre 3
pre 5
pre 7
pre 9
pre 11
pre 13
pre 15
pre 17
pre 19
Handling
post 2
post 4
post 6
post 8
post 10
post 12
post 14
post 16
post 18
post 20
Time
HEAD
EYE
COMB

Figure 12 (page 42) is a column chart comparing the total average Head, Eye and Comb temperatures of the 13 Hens with the total Average Head, Eye and Comb temperatures of the 6 Roosters across Pre measurement, handling and post measurement. The X axes displays the body part either the Head, Eye or Comb and the measurement period with P being pre measurement, H being handling and PO being post measurement. The Y axes displays the Average temperature recorded in degrees Celsius. The BLUE columns represent the overall averages for the Roosters while the RED columns represent the overall averages for the Hens.

Figure 12 shows a dramatic decrease in average Head, Eye and Comb temperatures of all 19 subjects from Pre Measurement to Handling. Hens Heads decreased by 3.3 degrees going from an average of 31.1 degrees during pre-measurement to an average of only 27.8 degrees during handling, Hens Eyes decreased by 2.75 degrees going from an average of 34.5 degrees during pre-measurement to an average of only 31.75 degrees during handling, Hens Combs decreased by 3.68 degrees going from an average of 28.8 degrees to an average of only 25.5 degrees. Roosters Heads decreased by 2.56 degrees going from an average of 31.26 degrees during pre-measurement to an average of only 28.7 degrees during handling, Roosters Eyes decreasing by 2.58 degrees going from an average of 34.8 degrees during pre-measurement to an average of only 32.48 degrees and their Combs decreased by 2.268 degrees going from an average of 28.8 degrees down to an average of only 26.6 degrees.

Paired samples *t* tests comparing the average Head, Eye and Comb temperatures at the pre measurement 20 minute mark with their corresponding average temperatures during handling for all 19 subjects returned significant results, *t(18)=13.212, p<0.05, t(18)=5.565, p<0.05 and t(18)=8.810, p<0.05* for the Head, Eye and Comb respectively.

Figure 12 also shows that for Hens, the Comb was the body part that saw the biggest temperature drop during the handling condition. For Hens the Comb temperature dropped from an Average of 28.87 (degrees Celsius) over the 20 minute pre measurement period to an average of only 25.9 (degrees Celsius) across the Handling period. As can be seen in *figure 12* the Head was the body part that saw the biggest temperature drop during handling for Roosters. Handling saw the average Head temperature for Roosters drop by 2.56 degrees from the Pre measurement average.

As can be seen in *figure 12* on average, Roosters Heads, Eyes and Combs are hotter than those of the Hens. The average pre measurement Comb temperatures are the exception in which both Roosters and Hens Combs sit at an average of 28.8 degrees.

Figure 12.

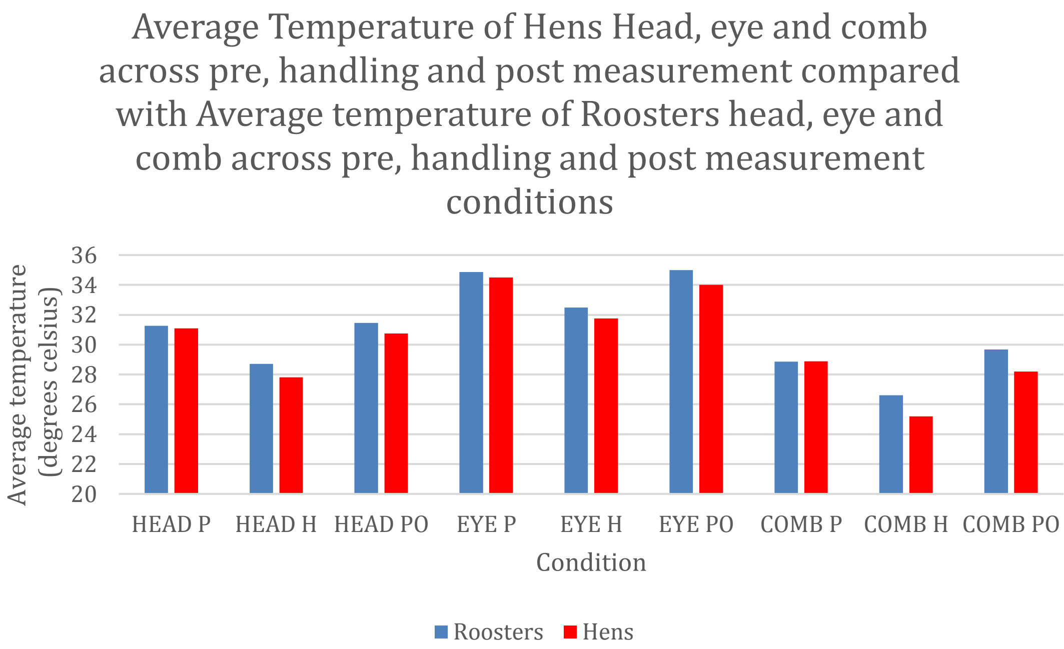
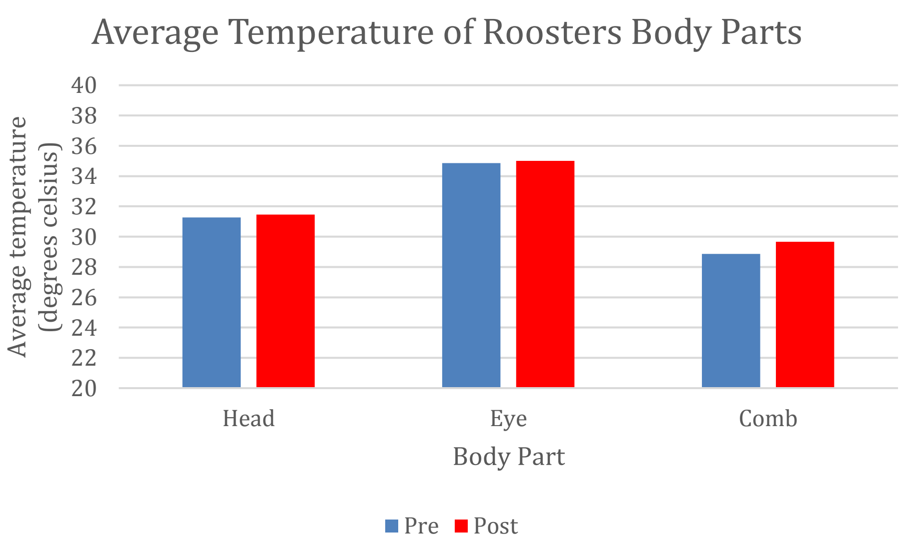


Figure 13 (pages 443/44) is 2 column charts comparing the combined average Head, Eye and Comb temperatures of 13 Hens and 6 Roosters in the Pre measurement period with the combined average Head, Eye and Comb temperatures of the 13 Hens and 6 Roosters in the Post measurement period. The X axes shows the Body part (Head, Eye or Comb) while the Y axes is the average temperature in degrees Celsius. The BLUE represents the average temperatures during PRE measurement while the RED represents the average temperatures during POST measurement.

The most consistent trend is the significant difference in pre and post measurement average Head, Eye and Comb temperatures for all subjects. Although this trend can be observed in the figure, Paired Sample *T* tests were carried out to compare average Pre and post measurement temperatures for the Head, Eye and Comb for all 19 subjects. No significant difference was found, *t(18)=.188, p>0.05, t(18)=1.507, p>0.05* and *t(18)=.384, p>0.05* for the Head, Eye and Comb respectively.

Figure 13.



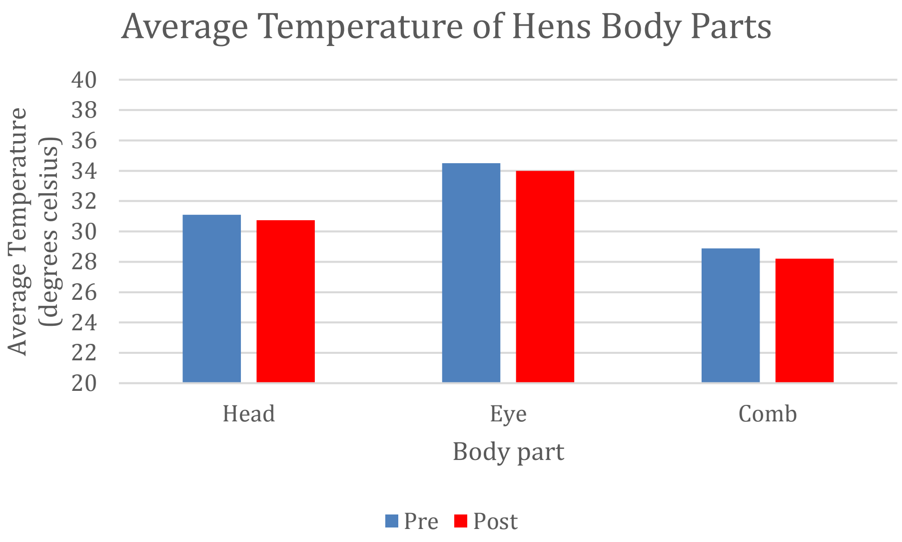


Table 1.

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	PRECOMB - HANDLINGC	3.98421	1.97126	.45224	8.810	18	.000

Table 1. Shows the results of a paired samples T Test between the average Comb temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Comb temperature taken during handling for all 19 subjects.

Table 2.

Paired Samples Test

		Paired Differences			t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Pair 1	PREEYE - HANDLINGE	3.54737	2.77836	.63740	5.565	18	.000

Table 2. Shows the results of a paired samples T Test between the average Eye temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Eye temperature taken during handling for all 19 subjects.

Table 3.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PREHEAD - HANDLINGH	3.62105	1.19470	.27408	3.04523	4.19688	13.212	18	.000

Table 3. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Head temperature taken during handling for all 19 subjects.

Table 4.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PRECOMB - POST20CO	.08421	.95467	.21902	-.37593	.54435	.384	18	.705

Table 4. Shows the results of a paired samples T Test between the average Comb temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Comb temperature at the post measurement 20 minute mark for all 19 subjects.

Table 5.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PREEYE - POST20EYE	.75263	2.17648	.49932	-.29640	1.80166	1.507	18	.149

Table 5. Shows the results of a paired samples T Test between the average Eye temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Eye temperature at the post measurement 20 minute mark for all 19 subjects

*Table 6***.**

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PREHEAD - POST20HEAD	.02632	.60904	.13972	-.26723	.31987	.188	18	.853

Table 6. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for all 19 subjects and the Average Head temperature at the post measurement 20 minute mark for all 19 subjects

*Table 7***.**

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PRECOMB - HANDLINGC	4.28462	2.21015	.61298	2.94904	5.62019	6.990	12	.000

Table 7. Shows the results of a paired samples T Test between the average Comb temperature at the pre measurement 20 minute mark for the 13 female subjects (Hens) and the Average Comb temperature taken during handling for the 13 female subjects (Hens).

Table 8.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	PREEYE - HANDLINGE	3.12308	.97139	.26941	2.53607	3.71008	11.592	12	.000

Table 8. Shows the results of a paired samples T Test between the average Eye temperature at the pre measurement 20 minute mark for the 13 female subjects (Hens) and the Average Eye temperature taken during handling for the 13 female subjects (Hens).

*Table 9***.**

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PREHEAD - HANDLINGH	3.95385	1.00051	.27749	3.34924	4.55845	14.248	12	.000

Table 9. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for the 13 female subjects (Hens) and the Average Head temperature taken during handling for the 13 female subjects (Hens).

Table 10.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PRECOMB - HANDLINGC	3.33333	1.23396	.50376	2.03837	4.62830	6.617	5	.001

Table 10. Shows the results of a paired samples T Test between the average Comb temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Comb temperature taken during handling for the 6 male subjects (Roosters).

Table 11.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PREEYE - HANDLINGE	4.46667	4.90333	2.00178	-.67906	9.61240	2.231	5	.076

Table 11. Shows the results of a paired samples T Test between the average Eye temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Eye temperature taken during handling for the 6 male subjects (Roosters).

Table 12.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	PREHEAD - HANDLINGH	2.90000	1.35056	.55136	1.48268	4.31732	5.260	5	.003

Table 12. Shows the results of a paired samples T Test between the average Head temperature at the pre measurement 20 minute mark for the 6 male subjects (Roosters) and the Average Head temperature taken during handling for the 6 male subjects (Roosters).

Discussion

The research was a direct replication of Edgar et al, (2013) who found that handling caused a significant decrease in Hens Combs surface temperatures and changes in the Head and Eye temperature. The aims of this research were to provide further support for the hypothesis that handling could cause stress in Chicken and that infrared thermal imaging could be used as a successful measure of stress as indicated by blood flow. For the purpose of this research the measure of 'stress' was taken as a significant decrease in surface (Head, Eye and Comb) temperatures of subjects as a result of stress-induced hyperthermia (SIH).

Hens

Pre measurement

Results show a steady rise in surface temperature of the 13 female subjects during the pre and post measurement periods. Hens Head, Eye and Comb temperatures were seen to rise slowly from the beginning of pre measurement and would consistently reach their hottest average temperature at the end of the 20 minute pre measurement period. This slow but steady increase would suggest habituation to the pre measurement. Although there was a three day acclimatization period where the subjects were exposed to the researcher and the equipment they did not experience being singled out (being the only subject followed by the researcher with an infrared thermal imaging camera). Being singled out in the group by the researcher may have caused some unease at the beginning of the pre-measurement phase. It is likely however that the unease settled and the subjects became habituated to being followed once it was established there would be no consequences or dramatic stimuli changes during the pre-measurement phase.

Handling

The results show a significant temperature decrease in all three body surface areas of Hens during the 5 second handling phase. This is consistent with the findings of Edgar et al (2013). This research takes stress-induced hyperthermia as a measure of 'stress' in subjects. Although stress-induced hyperthermia is usually classified as an increase of core body temperature of between 0.5 and 2 degrees Celsius (Edgar, et al, 2013) here a temperature change of at least 0.5 degrees Celsius caused by peripheral vasoconstriction is being used as a measure of stress-induced hyperthermia.

A decrease in surface area temperature can be used as a measure for stress-induced hyperthermia as the retention of heat needed to increase core temperature would cause a decrease in surface area temperatures. All surface area measures of the 13 female subjects decreased by between 2.26 degrees Celsius and 2.56 degrees Celsius during handling. All subjects experienced a surface area temperature decrease of more than 2 degrees Celsius which not only meets the requirements of stress-induced hyperthermia but exceeds them. All subjects experienced stress-induced hyperthermia following handling.

It is interesting to note that in Hens, the Comb experienced the most significant average decrease in temperature during handling, this finding is consistent with the findings of Edgar et al (2013). The Head was found to have a slightly smaller decrease during handling than the Comb but more significant than the Eye. Although still a significant decrease, the Eye was found to have the smallest average temperature decrease during handling which is not consistent with the findings of Edgar et al (2013) who found the Eye had a larger and more significant average decrease in temperature during handling Than the Head. The difference in findings could be due to the age and level of development of the subjects. As the subjects used in this research were all less than a year old and considerably younger than those used in Edgar et al (2013). Although the findings of this research and those found in Edgar et al (2013) are different the overall finding that all surface area measures saw a significant decrease during handling is consistent.

Post measurement

Edgar et al (2013) found that on average the Eye took approximately 10 minutes to return to baseline temperature while the Comb took approximately 15 minutes to fully recover from the stress-induced hyperthermia caused by Handling. It was hypothesised that the Head, Eye and Comb temperatures of the subjects would recover quicker in this research, and it was expected that they would return to baseline temperatures in less time due to the difference in methodology. In Edgar et al (2013) subject were placed in a holding box and removed from the group during baseline and handling while the 19 subjects in this research were never removed from the safety of the group. This was not the case, the impact of handling had an even longer effect on post measurement temperatures for the 13 female subjects in this research.

There was a significant difference in average body part temperatures obtained at the pre measurement 20 minute mark and those obtained at the post-measurement 20 minute mark. As can be observed in *figure 1* Hens not only experienced stress-induced hyperthermia but the Hens Head, Eye and Combs had not recovered from the stress-induced hyperthermia by the 20 minute post measurement mark. The Hens average Head Eye and Comb temperatures never returned to those obtained in pre measurement before handling. This may be have been caused by a change in the way subjects viewed their environment. The environment may have been viewed as ‘unsafe’ and the subjects may have been on edge waiting for another disruption or stressful event.

In Edgar et al (2013) each subject was removed from the housing pen for the handling and baseline measurement phases, this left the housing pen a 'safe', stable environment where the Hens were left alone potentially leading the subjects to feel 'safe' when they were returned to the housing pen and leading them to recover from the stress-induced hyperthermia more easily. Subjects in this research were never removed from the housing pen. Pre measurement, handling and post measurement phases were all carried out in the housing pen making their environment an unstable one, one where unexpected or stressful events can occur. It has been established that handling caused the subjects to experience stress, remaining in the environment where the stressful event occurred could have caused the subjects to remain in a state of unease and therefore the recovery period was longer.

Body parts

Throughout the research there was a clear temperature order for Hens body parts. The Eye was consistently the hottest body part throughout all phases. The Head was the next hottest body part leaving the Comb as consistently the coldest. The eye may be consistently the hottest body part due to being the smallest. The Head and Comb are larger than the Eye and the larger the body part the harder it is to retain heat. The Comb being the coolest body part was expected based on past research into the Comb suggesting it is the of the temperature regulator for the chickens body, remaining cooler than other body parts to prevent overheating in the animal (Homestead on the Range, 2015).

This order was consistent for all female subjects with the exception of subject Blue Dark Green. Subject Blue Dark Greens Eye was again the hottest body part throughout all phases but during pre-measurement and handling the Head and Comb were relatively equal temperatures. During post measurement the Head consistently reached a higher temperature than the Comb for subject Blue Dark Green. This is consistent with the findings that the Comb's temperature was most affected by handling. For all Subjects the Comb consistently had the largest decrease both during handling and from pre measurement to post measurement. The same trends were evident in subject Blue Dark Green.

These findings are not consistent with the findings of Edgar et al (2013) who found the Eye to have the lowest temperature throughout the research followed by the Head leaving the Comb to consistently have the highest temperature. It is possible that this difference was due to a difference in technology. Advances in infrared thermal imaging cameras and analysis programs have been made since Edgar et al (2013) research in 2013. The software may have allowed for a more accurate temperature reading of smaller body parts such as the Eye.

Results show temperatures for the Head, Eye and Comb remained relatively stable with very little variation during pre and post 20 minute measurement phases. Temperature measures for the 13 Hens during pre and post measurement showed very little variability in all subjects with the exception of subject Yellow Light Blue. This finding is consistent with the baseline measurement phase in Edgar et al (2013). The very small amount of variability in temperatures found for the majority of subjects suggests there were very few extraneous variables influencing the blood flow (stress) of the subjects throughout the measurement periods. This would suggest that the acclimatization period of only three days, compared to the seven used in Edgar et al (2013), was a sufficient amount of time for the subjects and the extra four days were unnecessary.

Subject Yellow Light Blue experienced some variability in temperature but the variability was consistent. The variation was not limited to a single time period, body part or measurement phase suggesting that the subject was generally more temperamental and easily phased compared to other female subjects and this finding was likely not a result of the measurement phases themselves.

All three surface area body parts chosen in this research (Head, Eye and Comb) appear to be useful body parts for future infrared thermal imaging research. They each provide successful measureable changes.

Roosters

Subjects Blue Purple and Yellow Dark Green experienced unexplained data points. Subject Blue Purple experienced a large spike in the Eye temperature at the 19 minute mark during pre-measurement and subject Yellow Dark Green experienced 2 unexplained spikes in Head temperature one in the 14^{th} minute of pre measurement and one in the 13^{th} minute of post measurement. As temperature spikes did not occur in the majority of subjects (the spikes only occurred one or twice in the 2 subjects, the spikes only occurred in one body part, were not consistent across all three body parts of the subjects, and are completely un explained by an event or stimulus) they were removed from the analysis and put down to either a human or equipment error and are not significant in terms of this research.

Pre measurement

Unlike the female subjects, the six male subjects displayed no steady rise in temperature throughout the pre measurement phase. Rooster's pre-measurement temperatures largely remained stable and consistent. This would suggest that unlike the female subjects in this research and those in Edgar et al (2013) the males either did not need to habituate to the pre measurement phase or they never became comfortable in the environment at all. Baseline temperature measures in the Roosters original housing environment would need to have been taken before the subjects were moved into the research environment in order to determine if the Roosters habituated during the acclimatisation phase or not at all. Without temperatures being obtained in the Roosters natural housing environment there is no way to determine which scenario is correct. The presence of the researcher and the research equipment in such close proximity may have caused Roosters temperatures to remain lower than they would be in a more natural environment consistent with how they were raised.

Handling

There was a significant drop in the Head and Comb temperatures for the six male subjects from the end of the pre measurement phase to the handling phase, which is consistent with the Female subjects both in this research and those in Edgar et al (2013). For Roosters the Head on average had the largest and most significant drop in temperature during handling. The Eye temperatures obtained at handling, although dropping at an average of more than that of the Comb, did not drop by a significant level from the temperatures obtained at the end of the 20 minute pre-measurement phase. The Comb temperatures obtained during handling were significantly lower than those obtained at the end of the 20 minute pre measurement phase.

The finding that the Head had the most significant temperature decreased during handling and the Comb had the smallest was surprising and one that is not consistent with the findings for the female subjects in either this research or Edgar et al (2013). With no equivalent research being carried out with Roosters it is hard to determine if this is due to a sex difference is subjects or if there is another explanation

Although Roosters average Eye temperature did not decrease at a significant level from pre-measurement to handling they did drop by an average of 2.38 degrees Celsius. All male subjects experienced surface area temperature decreases of more than 2 degrees Celsius during handling which not only meets the requirements of stress-induced hyperthermia but exceeds them. All subjects experienced stress-induced hyperthermia following handling, There was no fundamental difference between Hens and Roosters in the finding that handling causes stress.

Post measurement

A steady rise in temperature can be observed throughout the post measurement period as time after handling increases in only half of the subjects (Blue Purple, Blue Orange and Blue Light Blue). Subjects Yellow White, Yellow Dark Green and Yellow Purple do not have a steady trend in temperatures during the post measurement phase, there appears to be a large amount of variability in surface area temperatures. The variability in temperatures obtained in post measurement for the Roosters is not consistent with the post measurement trends of Hens. This difference may be due to Roosters being more temperamental than Hens. The male subjects may have found it more difficult to settle quickly after handling. The lack of similar research with Roosters makes it difficult to say for sure.

It is important to note that although there is no steady increase in temperatures for Roosters during post measurement as there is in Hens this does not mean that Roosters were more affects by handling or did not recover as well as Hens after handling. During handling Roosters average surface area temperatures dropped less than those of Hens. Roosters average surface area temperatures in post-measurement not only returned to those obtained in pre-measurement but exceeded them. This finding is similar to that of the Hens used in Edgar et al (2013) but not consistent with the findings of the 13 female subjects used in this research.

Roosters took on average approximately 10-15 minutes to recover from the stress-induced hyperthermia experienced during handling. This recovery time is consistent with the hens in Edgar et al (2013). Handling affected the blood flow of Roosters for an extended period of time.

Body parts

Overall there appears to be very little variability in Roosters Head, Eye and Comb temperatures throughout pre and post measurement periods. Pre measurement period for subject Yellow Dark Green is the exception. Subject Yellow dark Green may be more temperamental and easily affected by environmental changes and therefore need longer to habituate.

Three of the male subjects have a clear order of surface area temperature. The Eye is consistently the hottest body part with a clear distinction from the next hottest body part, the Head and another clear distinction between the temperatures of the Comb, the coolest body part for subjects Blue Orange, Blue Light Blue and Yellow white. As was the case with the 13 female subjects, the Eye was consistently the hottest body part throughout all measurement phases. In Male subjects Yellow Dark Green, Yellow Purple and Blue Purple the Head and Comb are not only equal for some time but the Comb periodically exceeded the temperature of the Head such as is the case with the pre measurement phase in subject Blue Purple. Any number of extraneous variables could be responsible for the change in Roosters surface area temperatures such as a perceived threat in the environment or a change in social hierarchy standings or it could simply be a trait of that animal to have subtle changes body temperature, we just don't know.

Although there is some variability in the temperatures of Roosters Head, Eye and Comb these body parts are still reliable and effective as temperature measures for this form of research.

Animal welfare

It is important to obtain and sustain the health and welfare of animals. Animal research is carried out to better understand animal welfare and how to improve it. This research focused on providing evidence that both handling causes stress in animals and that infrared thermal imaging is a reliable non-invasive way to measure this stress. Using infrared thermal imaging technology this research was successful in providing evidence that handling causes stress in Chickens.

The effects of stress on animals has been well documented (Dantzer et al., 1997, Martin et al,. 2011 and Moberg,. 2000) so the most significant finding of this research in terms of animal welfare is not that handling causes stress, but that the effects of stress on animals are long lasting. Hen's bodies had not recovered from the stress-induced hyperthermia by the end of the 20 minute post measurement phase. Stress does not only affect the mental health of animals but has a significant impact on the physical health of an animal as well. If 5 seconds of handling can cause over 20 minutes of stress-induced hyperthermia in Hens, then the detrimental effects of continuous stress on Hens could be irreversible.

Infrared thermal imaging

Infrared thermal imaging in this research and that of Edgar et al (2013) proved a useful means of data collection in Chickens. Infrared thermal imaging provided clear, valuable temperature measures of surface areas in both Hens and Roosters and evidence for stress-induced hyperthermia after handling. Infrared thermal imaging is a non-invasive means of obtaining relevant data in animal welfare research and would be beneficial in future animal research.

Limitations of research

There were several limitations experienced in this research. One of the biggest was the limited range of motion available with the camera. The camera was required to be plugged in to the experimental laptop at all times. This meant the researcher was restricted in movement to a minimal distance from the Laptop. Hens have a constant Head movement involved in natural behaviours such as pecking and walking. The constant Head movement paired with the limited range of the camera meant obtaining useable angles of the Head, Eye and Comb was extremely difficult.

In future research the use of a holding box would be beneficial. Placing the subjects in a holding box during measurement phases, as was the case in Edgar et al (2013), would increase the researcher's ability to obtain useable footage of the desired surface areas as the subjects movements are limited to a smaller area.

A further limitation was the different length of time lapses between recording sessions for each animal. A new recording session was required for each measurement phase for every animal. This meant the camera had to be reset and new atmospheric temperatures had to be taken before the start of pre-measurement, handling and post-measurement. There was no way to ensure the same amount of time was taken between measurement phases this time difference, especially between handling and post-measurement, could have influenced post-measurement findings in recovery time from handling.

It could be beneficial in future research to have a pre-arranged time frame between each measurement period. Although a time lapse between measurement periods would still be a limitation setting a timer for 10 minutes between measurement phases would at least allow the time between measurement phases for each subject to be the same and therefore limit variability between subjects.

There is always a risk of the influence of extraneous variables in any research. More than one handler was used in this research which could be considered an extraneous variable and a limitation. Two different handlers were needed due to scheduling conflicts with the main handler. The main handler was used for 17 of the 19 subjects while a secondary handler was used for 2 of the subjects. The use of a different handler, with a different handling technique, and one that was unfamiliar to the subjects could be an extraneous variable and may have influenced the subject's response to the handling. In future research it would be advised to use just one handler for each subject as there is no way to measure the impact a second handler could have on the findings.

Other potential extraneous variables include, the subjects proximity to other subjects during measurement phases, the environmental temperature, the ease of access to food, the subjects standings in the hierarchy and outside noise.

Further limitations were experienced in the analysis stage. One limitation observed in the analysis of the subject's surface area temperature was the change in number of pixels in a useable frame. As the subjects were continuously moving around a pen the subjects were always in a different proximity to the researcher (always within 1 metre). The differing proximity to the researcher meant a differing proximity to the camera and the further the subject was from the camera the smaller the surface area became. Due to the differing size of the surface areas there was a difference in the quality of the footage and number of pixels in any useable frame during analysis.

A more experienced researcher or more experience with the camera equipment would allow for better use of the zoom functions to prevent large differences. The finding that the majority of temperatures obtained throughout the measurement phases remained relatively stable suggests that the size of the body surface and number of pixels in the frame did not significantly affect the findings.

Conclusion

The aims of this research were to provide further support for the hypothesis that handling could cause stress in Chicken and that infrared thermal imaging could be used as a successful measure of stress as indicated by blood flow. Infrared thermal imaging was found to be a useful measure of stress-induced hyperthermia and blood flow in surface areas of both Hens and Roosters. The Head, Eye and Comb were found to be adequate measures of surface body temperature and will be useful in future research. All body surface areas showed evidence of stress-induced hyperthermia experiencing a decrease in temperature during handling. The finding that the effects of stress on Hens are long lasting is an important one for animal welfare research. Future research should focus on core body temperature as well as surface areas.

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