The Effects of Simulated Transportation Conditions on the Welfare of White-Feathered Layer Pullets

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Overall Abstract

This study evaluated the effects of various temperature (T) and relative humidity (RH) combinations, and durations (D), on the welfare of pullets during simulated transportation. In a 5x2 factorial arrangement (T/RH combinations, and D (three replications)) pullets (n=240) were randomly assigned to one of five treatments, 21°C 30%RH (21/30), 21°C 80%RH (21/80), 30°C 30%RH (30/30), 30°C 80%RH (30/80), and -15°C (-15), for a four or eight hour D. Pre-treatment, pullets were orally administered data loggers (recorded core body temperature (CBT)), which were retrieved post-treatment. Foot T, body weights, and blood samples were taken pre- and post-treatment. Video recorded during exposure and analyzed for behaviour. Post-treatment, pullets were slaughtered, and carcasses were analyzed for muscle tissue characteristics (pH, water-holding capacity, and colour). In a randomized complete block design, data were analyzed via Proc Mixed (SAS 9.4). Differences were determined at $P \leq 0.05$. Pullet CBT during the last hour of exposure was lowest in the -15 treatment. Pullet ΔCBT was greater in the eight hour D compared to the four hour D. Compared to the -15 treatment, pullets in 30/30 and 30/80 surveyed more, and pullets in 30/80 were more active. Pullets exposed to 30/30 and 30/80 panted more. Along with the 21/30 treatment, pullets in the -15 and 21/30 treatment spent more time motionless, compared to the 30/30 and 30/80 treatments. Pullets exposed to -15 preened less compared to 21/80 and 30/30. Foot T were lowest in the pullets exposed to -15, and higher in the pullets in 30/80 compared to the neutral and cold treatments. Final blood glucose levels were higher in pullets in 30/80 compared to 21/30 and 21/80. The partial pressure of CO_2, total CO_2, and bicarbonate levels were lower in pullets exposed for eight hours. Live shrink (kg) was higher in pullets exposed to 30/30 and 30/80 (compared to 21/80), and in the eight hour D (kg and %). No biologically significant impacts were found for pullet muscle tissue characteristics. Exposure to thermal stress required pullets to implement behavioural and physiological mechanisms to maintain homeostasis. A D of up to eight hours had minimal impacts on pullet well-being.

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-15	-15°C, uncontrolled RH
21/30	21°C and 30%RH
21/80	21°C and 80%RH
30/30	30°C and 30%RH
30/80	30°C and 80%RH
a*	redness
ACTH	adrenocorticotropic hormone
b*	yellowness
BEecf	base excess in the extracellular fluid
BW	body weight
Ca	calcium
cAMP	cyclic adenosine monophosphate
CBT	core body temperature
CIVD	cold induced vasodilation
CRH	corticotropin-releasing hormone
D	duration
DFD	dark, firm, dry
H/L	heterophil to lymphocyte ratio
HCO_3^-	bicarbonate
HPA	hypothalamic-pituitary-adrenal
Hz	hertz
K	potassium
kg	kilogram
L*	lightness
mmHg	millimeters of mercury
mmol/L	millimoles per liter
Na	sodium
pCO_2	partial pressure of carbon dioxide
PCV	pack cell volume

pO_2	partial pressure of oxygen
PSE	pale, soft, exudative
RH	relative humidity
sO_2	soluble oxygen
T	temperature
tCO_2	total carbon dioxide

1.0 Chapter 1. Literature Review: The effects of transportation conditions on the welfare of white-feathered layer pullets

1.1 Introduction

There has been a significant amount of public concern over the transportation of poultry due to the potential negative consequences on bird welfare (Altan et al., 2010). Pullets are essential to subsequent egg production. They are raised in commercial rearing facilities until approximately 17 weeks of age (Nielsen et al., 2011; NFACC, 2017). Afterwards, they are transported to a separate laying facility where they are kept for the remainder of their laying period (NFACC, 2017). Transportation of poultry can consist of various steps. First, producers must pre-plan the catching and loading processes to minimize the amount of time needed and handling required for catching and loading of birds (NFACC, 2017). Differing from broilers and spent laying hens, pullets are typically not feed restricted before transportation, as they are not used for subsequent meat production (NFACC, 2017). The light intensity in the barn is typically lowered during catching and loading, and birds are caught and generally placed in flat deck trailers using either a loose crate or cart system (NFACC, 2017). Catching and loading are performed by competent personnel, and the producers or a designated representative will oversee the process (NFACC, 2017). Any bird that is unfit for transportation (sick, injured, wet, or otherwise deemed unfit) will not be loaded (NFACC, 2017). The recommended maximum crating density for transportation of pullets and laying hens is 63.0 kg/m^2, but this density should be reduced by 15-25% during the summer months (NFACC, 2017). Once birds arrive at a destination, they are unloaded and placed in the new facility (NFACC, 2017). Rearing and laying facilities may be on the same site, however, these barns may also be at different locations, where pullets must be transported to the facility (NFACC, 2017). There is limited research on the impacts of transportation on pullets, as studies have mainly focused on the impacts of transportation on broilers, therefore extrapolation from previous research may be necessary. Transport of pullets is an essential component of the poultry industry, and care should be taken to avoid situations which can cause birds high amounts of stress, leading to high mortality rates or decreases in future production (Mitchell and Kettlewell, 2009; Nielsen et al., 2011).

There is a concern whether the environmental conditions present during transportation cause birds stress and negatively impact their welfare. During catching, crating, and loading, birds may be subjected to rough handling, which can be a significant stressor (Broom, 1988). Pullets can be exposed to various other stressors during transport such as the microclimate within

the trailer, noise, vibrations, social disruptions, and feed withdrawal (Mitchell and Kettlewell, 1998; Mitchell and Kettlewell, 2009; Koknaroglu and Akunal, 2013). Either heat stress or cold stress during transport is the primary cause of mortalities (Nielsen et al., 2011). Additionally, fear is a constituent of stress, and welfare can be reduced by chronic fear during transport (Altan et al., 2010). When transportation conditions are poor, the welfare of pullets will be compromised regardless of travel distance; however, during poor conditions, long transportation distances can exacerbate stressors (Mitchell and Kettlewell, 2009).

There are many ways in which stress can be assessed in birds subjected to transportation. Assessing the behaviour of birds during transport can be a useful tool to evaluate stress and fear. Birds will display various behavioural responses depending on whether they are too hot or too cold (Duncan, 1998). As well, physiological measurements can be used to quantify stress in pullets during transportation. These include core body temperature (CBT), live shrink (the amount of weight lost during the transport procedure), heterophil to lymphocyte (H/L) ratio, corticosterone, plasma glucose/electrolytes, hematocrit, hemoglobin levels, and levels of blood gases. Furthermore, various muscle tissue characteristics such as muscle pH, water-holding capacity, and colour, can be used to measure stress and are especially useful for assessing heat and cold stress. Exposure to heat or cold stress pre-slaughter can influence the rate of post-mortem glycolysis, which can result in a change in the pH of the muscle through the buildup of lactate, or the lack thereof (Pearson and Young, 1989; Honikel, 2004). A change in the pH can then result in a change in the muscle tissue colour as well as in the water-holding capacity, which can influence the muscle tissue drip, thaw, and cook loss (Pearson and Young, 1989). It is essential to collect data on numerous stress response measures, as transport will affect the animal in many ways. Quantifying various stress responses can aid in separating normal stress responses from those that reduce the welfare of the animal (Nielsen et al., 2011). This project aimed to expose pullets to hot and neutral temperatures, with a high and low humidity, as well as to a cold temperature that birds may experience during transportation, and to assess and quantify the stress responses of the pullets through behavioural, physiological, and muscle tissue characteristic measurements.

1.2 Core Body Temperature

The microclimate environment within the transportation truck may be a considerable stressor for poultry (Lin et al., 2006). Depending on ambient conditions and ventilation within the truck, temperatures within the crates can range from hot to cold and can influence the CBT of birds during transportation (Knezacek et al., 2010). Birds may implement behavioural and physiological mechanisms in an attempt to maintain homeostasis; however, when these mechanisms cannot stabilize the CBT, the birds may be at risk of hyperthermia or hypothermia depending on the crate temperatures (Knezacek, 2005; Lin et al., 2006). The average daily temperatures for June in Saskatoon, Saskatchewan, Canada between 1981 and 2010 were 15.8°C, with a maximum of 40.6°C and a minimum of -3.3°C (Environment Canada, 2019). A study by Knezacek (2005) observed that in a journey length of 175 minutes at an ambient temperature of 22°C with 24%RH, crate temperatures within a passively ventilated broiler trailer can range from 22-27°C (bird strain within crates not mentioned, and trailer curtains and vents were adjusted according to ambient conditions and driver discretion). When temperatures inside the crates are high, birds must exert energy to thermoregulate and maintain normal body temperature (Lin et al., 2006). In some instances, especially when humidity is high, thermoregulatory mechanisms may be ineffective, and the CBT will rise as a result of the inability to dissipate heat (Lin et al., 2005; Minka and Ayo, 2017). The normal body temperatures of birds at rest is approximately 41.5°C (Mount, 1979; Nicol and Scott, 1990). The critical upper temperature of poultry has been noted to be approximately 46-47°C, which can cause hyperthermia and death (Mount, 1979; Nicol and Scott, 1990). When the CBT reaches the upper critical limit, proteins in the body can denature and cells can no longer function, which together can result in organ dysfunction and death (Mount, 1979; Lin et al., 2006; Sherwood et al., 2013). Increases in the CBT of birds during transport are therefore very stressful and can severely impact the bird's well-being.

Exposure to cold conditions during transport can also negatively influence bird welfare. From 1981 to 2010 in Saskatoon, Saskatchewan, Canada, the average daily temperature in February was -12.5°C, with a maximum of 12.8°C and a minimum of -50.0°C (Environment Canada, 2019). Even at a low ambient temperature of approximately -18°C, crate temperatures (strain of birds within crates not mentioned) can range from -0.7 to 16.5°C (Knezacek et al., 2010). It has also been shown that at a low ambient temperature of -7°C, crate temperatures can be as high as 30.7°C inside the trailer (191-minute journey length) (Knezacek et al., 2010).

Though the use of tarpaulins during cold weather transportation can aid in protecting the birds, they can cause ineffective circulation of heat and moisture being trapped inside the trailer (Knezacek, 2005). When the CBT drops, it can be very stressful for the animal's body and may disrupt the proper functioning of core organs (Lin et al., 2006; Minka and Ayo, 2017). During cold stress, birds must also use energy to thermoregulate and maintain core temperatures (Lin et al., 2006). As well, when thermoregulatory mechanisms are ineffective, body temperatures may continue to drop. The lower critical temperature in which hypothermia and possibly death in domestic birds may occur is 19°C to 22°C (Mount, 1979; Nicol and Scott, 1990). Whether crate microclimates become cold enough to cause a decrease in the CBT of transported birds large enough to result in hypothermia is uncertain.

Several studies have observed changes in the body temperature of birds when exposed to either hot or cold temperatures. Sandercock et al. (2001) subjected broilers (35 or 63 days of age) to 32°C and 75%RH (heat stress) or 21°C and 50%RH (control) for two hours in a climatic chamber, and found that, at both ages, the rectal temperature of the birds was higher after exposure to heat stress. However, rectal temperatures may not be similar to the CBT of the birds. Menten et al. (2006) exposed broilers (42 days of age) to heat stress (35°C with 85%RH) for various durations (0, 30, 60, 90, and 120 minutes). Broilers exposed to the heat stress conditions for 90 and 120 minutes had the highest rectal temperatures (44.5°C and 45.0 °C, respectively), compared to broilers from the same flock that were exposed to normal conditions (details not mentioned) (Menten et al., 2006).

With regards to cold stress, Dadgar et al. (2010) subjected 39-42 day-old broilers to cold stress for three to four hours in a climate-controlled chamber and found that broilers exposed to -7°C had the lowest CBT (39.68°C), while broilers exposed to 15°C or 25°C had the highest CBT (40.46°C and 40.66°C, respectively). Dadgar et al. (2012b) also saw significant decreases in broiler (five to six weeks of age) CBT (35°C) when exposed to cold environmental conditions (-12°C) in a climate-controlled chamber for three hours, compared to broilers (41°C CBT) exposed to neutral conditions (22°C). Increases or decreases in the CBT during transport can negatively impact the animal's welfare and in extreme cases can lead to significant mortality. Therefore, the microclimate conditions within the truck during transportation should be taken into consideration.

1.3 Behavioural Responses

Observing and understanding behavioural changes of birds can be beneficial as it can be used as an indication of the bird's welfare (Duncan, 1998). Changes in behaviour can be indicative of the environmental conditions, thus can be used to determine if these conditions negatively impact welfare (Duncan, 1998; Mitchell and Kettlewell, 1998). When under limited amounts of stress, birds will display a variety of comfort behaviours, suggesting they are content in their environment. These comfort behaviours may include resting and/or preening. However, during transportation, birds are often subjected to various stressors and are confined within crates. Therefore, behaviours such as resting may be a result of the confinement, and preening can be a displacement behaviour in response to the stressful stimuli (Webster and Hurnik, 1990; Moberg, 2000). The first biological response to stress is changes in behaviour (Webster and Hurnik, 1990; Moberg, 2000). During transportation, birds will implement various behavioural changes aimed at maintaining a homeostatic body temperature.

When birds are exposed to cold temperatures, they display behaviours that aid in conserving body heat (Mount, 1979). Birds possess a highly insulated feather coat, which can aid them in combating cold ambient temperatures (Mount, 1979). Thermal insulation of the feathers can be increased through ptiloerection, which allows air to be trapped between the feather fibres (Mount, 1979). Additionally, birds will huddle and burrow under one another to reduce radiation and body surface area exposed to the environment, increasing conduction between birds (Mount, 1979; Strawford et al., 2011). Minimizing radiation and increasing conduction between conspecifics can ensure less body heat is lost to the environment, and body heat can be shared between individuals (Mount, 1979; Strawford et al., 2011). When changes in behaviour cannot stabilize the core body temperature of birds during transport, the implementation of further physiological mechanisms may be required (Moberg, 2000).

When birds during transport display behaviours during transport associated with cold stress and hypothermia, it can indicate poor environmental conditions, and poor welfare. Henrikson et al. (2018) observed that when turkey hens and toms were subjected to -18°C for eight hours, the birds spent significantly more time huddling (52%, and 30%), shivering (6%, and 2%), and performing ptiloerection (28%, and 57%), compared to those exposed to neutral conditions (20°C) for the same duration. Huddling and burrowing while in transport can also

shelter some birds from incoming drafts, which may reduce the impact of cold air on these birds (Strawford et al., 2011). Furthermore, in order to conserve energy and heat birds may remain motionless within the transportation crates (Minka and Ayo, 2017). When changes in behaviour in response to cold stress are unable to stabilize the CBT of birds during transport, it can put them at risk for hypothermia (Mount, 1979; Minka and Ayo, 2017). It has been noted that in a transportation journey lasting one to four hours with an ambient temperature of -22°C, the average temperature within the trailer (tarpaulins closed and one roof vent open) ranged from -19°C to 13°C (Burlinguette et al., 2012). Therefore, even during transportation in cold ambient conditions, the temperature within the trailer can be above the ambient temperature.

Heat stress can be another significant stressor for poultry during transport. Birds exposed to heat stress will display behaviours aimed to dissipate heat (Mount, 1979). The first behavioural responses that birds will implement are non-evaporative heat stress behaviours (Mount, 1979; Freeman, 1984; Weeks et al., 1997). Birds become more active and increase space usage within the crate to expose a higher body surface area to the environment (Mount, 1979). This can increase heat loss through radiation; however, due to the confinement of birds within a crate, movement and heat loss through radiation is very limited (Weeks et al., 1997). Additionally, birds may move away from one another to reduce conduction; however, this can also be very limited due to crate confinement (Mount, 1979; Weeks et al., 1997). Lastly, air flowing over the skin and plumage can aid in heat loss (Mount, 1979; Weeks et al., 1997; Lin et al., 2005). Convection during transport is aided by ventilation and air passing over the birds while the vehicle is in motion (Weeks et al., 1997). Again, in order to maximize heat loss through convection, birds may increase movement within the crate to disperse from conspecifics and increase body surface area exposed to the environment, though this is very limited (Mount, 1979; Weeks et al., 1997). Once the ambient temperature within the crate reaches the birds' body temperature, non-evaporative heat loss mechanisms may no longer be effective (Freeman, 1984). Therefore, birds must rely on evaporative heat loss to dissipate heat and maintain body temperature (Mount, 1979; Lin et al., 2005). Evaporative heat loss is the primary way in which birds dissipate heat when exposed to hot ambient temperatures (Lin et al., 2005). During evaporative heat loss, birds will increase their respiratory frequency (panting) so that the continuous movement of air over the upper respiratory tract will vaporize water into the air (Mount, 1979; Lin et al., 2005; Menten et al., 2006). Panting can be a valuable way to dissipate

heat; however, evaporative heat loss becomes ineffective once the humidity in the environment becomes high, as vaporization of water to the environment is unable to occur (Mount, 1979; Weeks et al., 1997). When ambient temperatures are high with high humidity, heat loss becomes inefficient, putting the birds at risk for hyperthermia (Freeman, 1984; Weeks et al., 1997). During transport, crate temperatures can be close to 30°C, even during negative ambient temperatures, which can pose a significant risk for the animals (Knezacek et al., 2010). It can be expected that during heat stress there would be an increase in activity and movement within the crate, as well as an increase in panting, as the birds attempt to dissipate heat and maintain homeostatic body temperature (Strawford et al., 2011). Though the performance of the mentioned behaviours can aid in dissipating heat and maintaining CBT, they can have a negative impact on the physiology of birds (Mount, 1979; Sherwood et al., 2013).

Besides thermal stress, fear-inducing stimuli such as noise, vibrations, social disturbances, and handling can also be stressful for birds during transport (Abeyesinghe and Wathes, 2001). As a response to handling during catching and loading, birds may increase vocalization rate and pitch (Barri, 2008). In the case of social disturbances, birds are confined within crates during transportation, creating small social groupings. This social disruption and confinement can cause frustration and aggressive pecking in birds (Siegel et al., 1978; Barnett, 2007). Additionally, vibrations and noise can be aversive for birds during transportation. As a response to these stressors, birds may increase activity in an effort to escape from these stimuli (Minka and Ayo, 2009). As well, birds may display more vigilance, in which they will survey the environment for incoming stressors (Rushen, 2000).

An increased duration may exacerbate any thermoregulatory and stress responses performed during transportation (Carlisle, 1998). Long transport durations can exacerbate the effects of heat stress, cold stress, and/or fear-inducing stimuli that birds may experience (Carlisle, 1998). As a result of performing thermoregulatory behaviours for a longer period of time, as well as longer durations exacerbating dehydration, the physiology of birds may be affected (Mount, 1979; Sherwood et al. 2013). Additionally, poor transportation conditions and longer durations may cause birds to become fatigued. Despite this evidence, the effect of increased transportation duration on time spent performing various behaviours during transportation is not well understood.

1.4 Extremity (Comb and Foot) Temperatures

As a result of exposure to cold or hot temperatures, the feet and combs can either succumb to frostbite or can be used to dissipate heat (Midtgard, 1989). Because these extremities are devoid of feathers, they can become susceptible to frostbite during cold temperatures (Rector et al., 1952; Midtgard, 1989). Birds have several mechanisms that enable them to cope with exposure to cold temperatures, such as cold-induced vasoconstriction or vasodilation (Rector et al., 1952; Midtgard, 1989; Wellehan, 2014). When birds are exposed to cold temperatures and are working to maintain optimal CBT, the body's immediate response is to vasoconstrict the blood vessels to the extremities to preserve heat for the vital organs (Millard, 1974; Wellehan, 2014). During prolonged exposure to severely low temperatures, the vessels to the extremities will periodically vasodilate to send increased blood flow to the extremities (Millard, 1974; Wellehan, 2014). This periodic vasodilation is known as cold-induced vasodilation (CIVD) and is a response aimed to preserve the extremities during cold exposure (Midtgard, 1989; Wellehan, 2014). It has been suggested that CIVD acts through the sympathetic nervous system and involves cholinergic vasodilation fibres and the arteriovenous anastomoses, which, when open, shunt blood to the extremities; however, these mechanisms have only been suggested in humans and are not well understood in poultry (Edwards, 1965; Millard, 1974; Daanen, 2003; Walloe, 2016). As cooling of the extremities continues, the periodic vasodilation will cease, and this is when frostbite can occur (Wellehan, 2014). This mechanism for frostbite has been found to occur in the feet of pigeons, ducks, chickens, and fulmars (Wellehan, 2014). Frostbite is defined as a localized injury to tissue due to both the cooling and thawing of the tissue (Wellehan, 2014). The freezing temperature of human finger skin is approximately -2.2°C due to the salinity of the human body fluids (Lewis, 1926; Goldman and Molnar, 1976; Nagpal and Sharma, 2004). However, the rate to which skin freezes can depend on various environmental factors, such as the air moisture, content air movement over the skin, and altitude (Lewis, 1926; Goldman and Molnar, 1976; Nagpal and Sharma, 2004). A high air moisture content, increased air movement over skin, and a higher altitude can all be contributing factors which accelerate the freezing rate of skin (Nagpal and Sharma, 2004).As the tissue freezes, ice crystals form within the cells, which can cause cell death by tearing and rupturing the cell membranes (Nagpal and Sharma, 2004; Wellehan, 2014). Frostbite can cause loss of proprioception in the affected area, pain, and potentially self-mutilation as a result of the pain (Wellehan, 2014). For tissue that has survived

frostbite, further long-term complications can include increased susceptibility to cold injury, loss of sensory ability in the tissue, and osteoarthritis in the extremity (Wellehan, 2014).

Frostbite in birds exposed to cold temperatures during transportation has not been well studied; however, the vasoconstriction and vasodilation responses have been shown in various bird species. A study by Millard (1974) studied the blood flow to the dorsal artery in the foot of the giant petrel *(fulmar Macronectes giganteus)* when immersed in ice water (-2°C). It was found that the blood flow in the dorsal pedal artery increased sharply (40-100 ml/min) during the first 10-15 second after cold immersion, which can signify rapid vasodilation (Millard, 1974). Afterwards, the blood flow dropped slightly for the next 10-60 seconds and then plateaued, which indicates vasoconstriction (Millard, 1974). During prolonged immersion in the ice water, periodic vasodilation occurs (approximately every three minutes) in the dorsal pedal artery, showing a cold-induced vasodilation response in the foot (Millard, 1974). It should be noted however, that the giant petrel is an Antarctic bird, thus they would have a greater adaptation to cold than would the domestic chicken. Therefore, extrapolation from the mentioned study above may be difficult. It has been found that at an ambient temperature of -22°C, crate temperatures inside the transportation truck may reach as low as -21°C (Burlinguette et al., 2012). More work is needed to fully understand how exposure to cold temperatures during transportation may cause cold injury to the feet and combs of pullets and the long term effects of these injuries.

Bare skin on the feet and combs can also act as a thermoregulatory mechanism for birds, as the tissue can dissipate heat to the environment when the body temperature increases (Richards, 1971; Midtgard, 1989). Dissipation of heat through the skin is the first thermoregulatory mechanisms of the animal when the CBT becomes too high (Whittow et al., 1964; Richards, 1971). For this mechanism to work, the blood vessels to the extremities will vasodilate, allowing for increased blood flow, which can reduce the body temperature of the bird (Whittow et al., 1964; Richards, 1971). When heat dissipating through the skin is inadequate to maintain homeostatic body temperature, the second mechanism for heat loss is through evaporative cooling (Richards, 1971). A study by Richards (1971) exposed hens to an ambient temperature of 30°C for one hour and found that the comb and toe temperatures increased by approximately 15°C. Richards (1971) also noted that when exposed to an ambient temperature between 26-35°C, the thermal index (estimate for the increase in skin temperature measured

through an ultrasound scan) for the naked skin of the combs and toes rapidly increased. Whittow et al. (1964) exposed hens to an ambient temperature of 23°C for 200 minutes and saw a similar increase in the temperatures of the birds' combs. During transportation, depending on ambient temperatures and truck ventilation systems, temperatures within a truck can reach near and above 30°C (Knezacek et al., 2010). These authors found that at an ambient temperature of -7.1°C, crate temperatures near the core of the front of the trailer during a transport journey that lasted 191 minutes reached between 29-30°C (Knezacek et al., 2010). Another study conducted by Dadgar et al. (2010) found that on a transportation journey lasting three to four hours, with an ambient temperature of 11°C, the temperature of the air within the crates reached 30°C. Therefore, these studies demonstrate that even during cold ambient temperatures, the crate temperatures can still be high enough where birds may need to implement thermoregulatory mechanisms, resulting in increased extremity temperatures. More research is required to fully understand the impact of hot crate temperatures on the comb and feet temperatures of birds, and the potential consequences of prolonged high skin temperatures.

1.5. Blood Parameters

1.5.1 Corticosterone

The level of corticosterone in the blood can be a physiological measure of stress in animals (Barri, 2008). The hypothalamic-pituitary-adrenal (HPA) axis is one of the first pathways to respond to stress. As a result of an incoming stressor, the hypothalamus will release corticotropin-releasing hormone (CRH), which will stimulate the anterior pituitary to release adrenocorticotropic hormone (ACTH) (Barri, 2008; Sherwood et al., 2013). Subsequently, ACTH will act on the adrenal cortex of the adrenal glands through the cyclic adenosine monophosphate (cAMP) pathway, to stimulate the release of corticosterone (Barri, 2008; Sherwood et al., 2013). Elevated levels of corticosterone can have various effects on the body, such as increasing concentrations of blood glucose through glycogenolysis and gluconeogenesis and suppressing the immune system through decreasing the production of white blood cells and inflammatory chemical mediators (Barri, 2008; Sherwood et al., 2013). The above effects function to increase the body's available energy to respond to stressors and maintain physiological homeostasis (Sherwood et al., 2013). However, corticosterone levels are regulated by a negative feedback mechanism which involves the hypothalamus and the anterior pituitary

(Saltzman et al., 2004; Sherwood et al., 2013). Secretion of corticosterone is dependent on the release of CRH and ACTH, therefore, when stressors are removed, CRH and ACTH production will decline, resulting in a decreased production of corticosterone (Sherwood et al., 2013). Additional factors such as circadian rhythms can influence corticosterone levels, with levels generally being highest in the morning and lowest during the night (Sherwood et al., 2013; Scanes, 2016). With regards to transportation, corticosterone can increase as a result of various non-specific stressors such as the presence of humans, handling, crating, social disturbances, vibrations and noise, and thermal stress. Therefore, due to corticosterone responding to various stressors, and being influenced by diurnal rhythms and negative feedback mechanisms, care should be taken when assessing levels of corticosterone in response to stress.

Various aspects of transportation, such as thermal stress, handling, vibrations, and transport, have been shown to influence levels of corticosterone (Beuving and Vonder, 1978; Broom, 1988). Beuving and Vonder (1978) cannulated the wing vein of White Leghorn (31 weeks old) laying hens (given 38 hours recovery period) and exposed the hens to 37°C and 25%RH for one week. It was found that as a result of heat stress, levels of corticosterone were elevated after one hour and 15 minutes of exposure (3.1 vs 5.3 ng/ml), but gradually decreased over the remainder of the exposure duration (Beuving and Vonder, 1978). Though the exposure duration to heat stress in this study is considerably longer than would occur during transport, elevated levels of corticosterone were noted after only one hour and 15 minutes of heat stress (Beuving and Vonder, 1978). Furthermore, on a separate group of cannulated White Leghorn hens (33-37 weeks old) Beuving and Vonder (1978) assessed the corticosterone response to immobilization via human hand for either 7.5 minutes, one hour, or to repeat handling (repeated immobilization for five minutes every two hours, over five days). With regards to birds immobilized for 7.5 minutes, it was found that corticosterone levels significantly increased after 45 seconds (using a student t-test) of immobilization (Beuving and Vonder, 1978). Birds immobilized for one hour had a significant initial increase in corticosterone, with smaller increases in corticosterone over the remained of the immobilization period (Beuving and Vonder, 1978). Finally, with regards to repeated handling, there was a significant increase in corticosterone after the first period of handling, but there was no significant decrease in corticosterone levels during the remainder of the experiment (Beuving and Vonder, 1978). Vibrations during transportation can also be a stressor for poultry. Carlisle (1998) placed 41-day

old broilers (fasted three hours prior) in transport crates (20°C crate temperature) and subjected the birds to various vibration frequencies (two, five, or ten Hz) for three hours. It was observed that those exposed to either five or ten Hz had a higher level of corticosterone directly post-exposure (Carlisle, 1998). A study by Zhang et al. (2009) subjected commercial broiler chickens (46 days of age, not feed restricted) to various transportation and lairage durations (27°C and 76%RH crate conditions). It was found that birds transported for 45 minutes and given a subsequent 45 minute lairage period had the highest levels of corticosterone (37.36 ng/ml) compared to broilers transported for 45-minutes and given a 3-hour lairage period (32.89 ng/ml), and compared to broilers that were not transported (33.38 ng/ml) (Zhang et al., 2009). Finally, Cheng and Jefferson (2008) subjected 17-week old laying hens to two hours of transportation. The laying hens were not feed restricted prior to transportation, and environmental conditions in this study were not mentioned (Cheng and Jefferson, 2008). Cheng and Jefferson (2008) observed that corticosterone levels in the hens subjected to transportation were higher (6.15 ng/ml) compared to those that were not transported (2.16 ng/ml). The previously mentioned studies, therefore, indicate that levels of corticosterone can change as a result of the various stressors that may occur during transportation.

1.5.2 Heterophil to Lymphocyte Ratio

Variations in the heterophil to lymphocyte (H/L) ratio can be another valuable measure of non-specific chronic stress in poultry (Felver-Gant et al., 2012). Responses in the immune system are highly influenced by stress through increases in corticosterone in the body (Thaxton and Puvadolpirod, 2000). As a result of birds being exposed to stressors during transport such as thermal stress, vibrations, noise, feed withdrawal, and/or social disruptions, corticosterone will increase in the body through the HPA axis (Thaxton and Puvadolpirod, 2000; Barri 2008; Sherwood et al., 2013). Increases in corticosterone will result in an increase in the H/L ratio by changing the levels of the heterophils and/or the lymphocytes produced in the body (Blecha, 2000; Elsayed, 2014).

Studies have been consistent in observing an increase in the H/L ratio of birds exposed to either heat stress or cold stress. A study by Altan et al. (2010) exposed broilers (35-36 days of age) to 38ºC for three hours within a floor pen and noted that the H/L ratio increased from 0.24 to 0.42 post-exposure. Furthermore, Campo and Prieto (2008) exposed different Spanish breeds

of chickens (Buff Prat and Red Barred Vasca, 24 weeks of age) to cold stress (0-10ºC) for a 12 week period, and noted a significant increase in the H/L ratio compared to those exposed to the control conditions (16 – 20°C) for the same duration. Other transport-related stressors can also cause changes in the H/L ratio of birds. In a study conducted by Bedanova et al., (2014), 42 day old broilers were crated and left for various durations (non-crated (control), two, or four hours) within the barn (conditions within the crate, 24.5°C and 40%RH). After the respective duration, birds were placed in a home pen, and blood was sampled at zero and 24 hours post-exposure, and it was noted that regardless of crating duration, the H/L ratio was higher when it was sampled 24 hours post-exposure (0.41 vs 0.33) (Bedanova et al., 2014). A study conducted by Compendio et al. (2016) feed restricted 16 week old pullets for seven hours, then crated (1.0 x 0.6 x 0.3 m, ten birds per crate) and exposed them to vibrations (one horsepower and 1800 rpm) for various durations (zero, two, four, and six hours), using a table attached to soft springs with an actuating system. Conditions within the chambers containing the vibration system were not mentioned in this study. It was found that pullets transported and exposed to vibrations for six hours had consistently higher H/L ratios compared to pullets who were not transported (control). It was noted that H/L ratios were not only increased one hour after exposure to simulated transportation and vibrations but remained higher 20 hours after exposure to treatment conditions. Lastly, a study conducted by Elsayed (2014) exposed 42 day-old broilers to various environmental temperatures (10-20°C, 15-25°C, and 25-35°C), for various durations (15, 50, or 150 kilometers), and found that the H/L ratio was higher for broilers exposed to 25-35°C. The ratio was also higher for those transported for either 15, 50, or 150 kilometers compared to the control group, which was not transported. Previous research has shown a consistent increase in the H/L ratio as a result of exposure to various stressors, and therefore the H/L ratio can be used as a reliable physiological indicator of stress in pullets.

1.5.3 Plasma Electrolytes

Levels of plasma electrolytes in birds can change as a response to stressors during transportation (Khosravinia, 2017). The conventional electrolytes studied as a response to stress are potassium (K), calcium (Ca), and sodium (Na) (Khosravinia, 2017). These electrolytes all have important functions in the osmotic and acid-base balance of the body and are important in the proper nerve and muscle function (Ahmad and Sarwar, 2006; Khosravinia, 2017). Both Na and K are essential in the resting and action potential of nerves in the body (Romero and

Whittam, 1971). On the other hand, Ca is important in the release and transmission of neurotransmitters between synapses of neurons (Meech, 1978). During transportation, due to dehydration and defecation, hyponatremia (low levels of Na in the blood) can occur as a result of a loss of water and electrolytes (Na) from the body with no replenishment (Thomas et al., 2008). Hyponatremia will cause metabolic alkalosis, which will result in an increased blood pH due to hydrogen ions diffusing from the blood into the interstitial fluid (Siggaard-Andersen and Fogh-Andersen, 1995; Thomas et al., 2008). Dehydration and hyponatremia can be exacerbated by long transport durations (Khosravinia, 2017). Thus, as a result of transportation, it can be expected that plasma electrolytes will decrease.

Studies examining plasma electrolyte variations in response to thermal stress are very limited; however, results from existing studies have been consistent (Khosravinia, 2017; Minka and Ayo, 2017). When broilers chicks were subjected to a transportation distance of 1000 kilometers directly post-hatch (27-29ºC with 35-46% RH, within the trailer), plasma levels of K, Ca, and Na decreased, compared to birds that were transported for only 200 kilometers. Chicks during transport may have a different response, as they will have access to yolk stores, which may influence levels of plasma electrolytes (Khosravinia, 2017). Additionally, 17-week old pullets that were transported in cold conditions as part of a preceding experiment (details not mentioned) were submitted to a clinic for analysis of blood physiology (Minka and Ayo, 2017). Compared to the baseline levels obtained prior to the preceding experiment, plasma levels of Na, K, and Ca were lower after transportation in the cold conditions (Minka and Ayo, 2017). Though there are limited studies on plasma electrolyte responses to stress, available results show a consistent decrease in Na, Ca, and K levels. Therefore, plasma electrolyte levels can be used as a measure of well-being in pullets subjected to transportation but should be coupled with other physiological measures of stress and well-being for more reliability.

1.5.4 Plasma Glucose

Levels of plasma glucose are a commonly used physiological indicator of stress and energy usage during transportation (Minka and Ayo, 2009). Glucose is an important fuel source for the body and provides the animal with the energy necessary to respond to stress and to perform necessary behavioural and physiological responses to maintain homeostasis (Zhang et al., 2009; Hernawan et al., 2012). In response to stressors, the animal's body will produce

increased amounts of glucocorticoids such as corticosterone and epinephrine (Sherwood et al., 2013). Corticosterone and epinephrine play an important metabolic role in mobilizing available glucose for animals attempting to cope with stress. Initially, the birds will utilize the glucose in their blood as a source of energy, as it is readily available for use (Sherwood et al., 2013). Birds are typically feed restricted before transportation, so there will be no new incoming nutrients into the body (Zhang et al., 2009). Therefore, once available blood glucose has been depleted, corticosterone and epinephrine will cause various metabolic effects which will increase the levels of glucose in the blood which animals can then use for energy (Matteri et al., 2000; Zhang et al., 2009; Sherwood et al., 2013). Increased epinephrine will cause higher rates of glycogenolysis from the carbohydrate and fat stores, which breaks down glycogen into glucose, and this glucose is then released into the blood (Zhang et al., 2009; Sherwood et al., 2013). Furthermore, increased levels of corticosterone will stimulate gluconeogenesis in the liver, where non-carbohydrate sources will be converted to carbohydrates which can be broken down to increase concentrations of blood glucose (Matteri et al., 2000; Sherwood et al., 2013). Therefore, both the increase in gluconeogenesis and glycogenolysis will cause an increase in the level of blood glucose over time, and the birds will be able to use this glucose to respond to stressors (Matteri et al., 2000; Sherwood et al., 2013). As a result of stress, it would, therefore, be expected that blood glucose levels would initially decrease as they are being used up, and then levels would increase as stores are mobilized. Blood glucose stores will be mobilized until homeostasis is achieved, and levels of corticosterone and epinephrine in the body decrease (Sherwood et al., 2013).

Results from various studies have consistently shown that as a result of stressors during transportation, such as thermal stress and feed restriction, plasma glucose levels will decrease over time (Vosmerova et al., 2010). Additionally, as a result of longer transportation durations, birds will have been feed restricted for a longer period of time, which can also cause a decrease in plasma glucose (Warriss et al., 1993; Vosmerova et al., 2010; Khosravinia, 2017). A study by Zhang et al. (2009) noted a significant decrease in plasma glucose for broilers that were transported for three hours (11.21 mmol/L) at 27°C, compared to broilers that were not transported (13.08 mmol/L), and broilers that were transported for only 45 minutes (12.56 mmol/L). The broilers in this study were not feed restricted before transportation, therefore, exposure to heat stress (27ºC) for a longer period of time may have required the birds to use more glucose (Zhang et al., 2009). Many studies have also consistently shown that cold exposure

reduces blood glucose. A study by Dadgar et al. (2012b) observed that the blood glucose levels in six-week old broilers exposed to cold conditions (-12°C) for three hours, were significantly lower (6.9 mmol/L) compared to birds exposed to neutral temperatures (22°C) for the same duration. Furthermore, another study by Dadgar et al. (2012a) exposed broilers to various temperatures for a three-hour duration and found that the blood glucose was lowest in broilers exposed to -14ºC (7.4 mmol/L), compared to broilers exposed to 21°C (10.8 mmol/L). Lastly, Dadgar et al. (2011) showed that, compared to control birds (exposed to 21°C for three hours), birds exposed to cold temperatures (-15°C for a three-hour duration) had significantly lower blood glucose levels (10.8 vs. 7.32 mmol/L). These studies can indicate that the birds were depleting the available blood glucose but had not yet mobilized stores from the body to increase the glucose in the blood. Unlike pullets, broilers are feed restricted before they are transported for slaughter. This means that pullets may have more energy and a higher initial blood glucose levels to respond to stress, compared to broilers. Nonetheless, plasma glucose can still be a useful measure of stress and fatigue in pullets after exposure to stressors during transportation.

1.5.5 Hematocrit and Hemoglobin

Measuring the hematocrit and hemoglobin levels of birds subjected to transportation can be used as a measure of stress. Hematocrit is the volume of packed blood cells compared to the total blood volume, expressed as a percentage (Bergoug et al., 2013). As a result of transportation, and especially during long durations, pullets may become dehydrated (Bergoug et al., 2013). Due to dehydration, the amount of fluid in the blood will decrease, causing the number of red blood cells per volume of blood to rise, increasing the hematocrit value (Bergoug et al., 2013). However, other studies have suggested that the hematocrit and hemoglobin levels will decrease due to hemodilution during evaporative heat loss as a result of heat stress (Ait-Boulahsen et al., 1989; Borges et al., 2004; Scanes, 2016). Hemodilution occurs as an adaptive response to heat stress that allows water loss through evaporative measures from the extracellular fluid instead of through the plasma, leading to a possible decrease in the hematocrit and hemoglobin (Borges et al., 2004). Thus, changes in the hematocrit and hemoglobin levels can be indicators of heat stress in birds; however, they should be coupled with other measures of heat stress for more reliable conclusions.

Variations in hematocrit and hemoglobin values due to thermal stress have not been well studied but results between studies have been consistent. A study by Borges et al. (2004) observed a decrease in the hemoglobin levels (7.25 vs. 7.75 g/dL) of 44-day old broilers after being exposed to 41°C in a temperature-controlled room for six hours. However, broilers in this study were given electrolyte supplements prior to exposure and were given access to feed and water during heat exposure (Borges et al., 2004). This may have influenced the study results as hemoglobin levels change as a result of dehydration. Ait-Boulahsen et al. (1989) feed restricted broilers for various durations (0, 24 or 72 hours) and subjected them to concurrent heat exposures (29°C to 37°C for four hours, and 37°C to 41°C for four hours) in a controlled chamber. This study found that heat stress had a significant effect on the packed cell volume of broilers, with birds fasted for 24 hours having a lower packed cell volume (27.8) compared to those that were not fasted (29.1) (Ait-Boulahsen et al., 1989). Hematocrit and hemoglobin values can be used in pullets to aid in indicating heat stress in birds during transportation, however, the relationship between hemoglobin, hematocrit, and thermal stress still is not fully understood (Scanes, 2016).

1.5.6 Blood Gases and Blood pH

Evaporative heat loss is the primary mechanism for poultry to dissipate heat when exposed to hot temperatures (Mount, 1979). Excessive panting can be dangerous for birds as it can affect the levels of blood gasses, which can, in turn, affect the acid-base balance in the body (Mount, 1979; Siggaard-Andersen and Fogh-Andersen, 1995; Sherwood et al., 2013). Carbon dioxide (CO_2) is the main gas in the blood that can be affected by hyperventilation, and it can be assessed through measuring the partial pressure of CO_2 (pCO_2) and the total CO_2 (tCO_2) (Sandercock et al., 2001). The partial pressure of CO_2 in the blood can be defined as the pressure exerted by CO_2 within the mixture of gases that are in the blood (Sherwood et al., 2013). Total CO_2 can be defined as including all forms of carbon dioxide within the blood, including bicarbonate, dissolved CO_2, and carbonic acid (Lumb, 2000; Sherwood et al., 2013). As a result of hyperventilation, there will be an increased elimination of CO_2 from the body, which will cause a decrease in the blood pCO_2 resulting in hypocapnia or respiratory alkalosis (Koelkebeck and Odom, 1995; Lumb, 2000; Sandercock et al., 2001; Sherwood et al., 2013). Due to a lower blood pCO_2, bicarbonate in the blood will also decrease in order to adjust to the new pCO_2 in the blood (Sherwood et al., 2013). As a consequence of a lower pCO_2, a buffer system in the blood

will be created resulting in an increase in the pH of the blood (Ahmad and Sarwar, 2006; Sherwood et al., 2013).There has been very little research conducted on the effect of thermal stress on levels of tCO_2 in poultry, therefore it is not well understood.

Though hyperventilation can affect the pCO_2 in the blood, it may have no effect on either the partial pressure of oxygen (pO_2) or soluble oxygen (sO_2) in the blood (Lumb, 2000). The pO_2 is the pressure exerted by O_2 in the blood, whereas sO_2 is the total amount of dissolved oxygen in the blood (Lumb, 2000; Sherwood et al., 2013). The body tightly regulates oxygen in the blood; therefore, a change in blood pO_2 and sO_2 may only be achieved if the air being inspired has a higher or lower concentration of O_2 (Lumb, 2000). Thus, the respiratory alkalosis resulting from panting is primarily based on the decrease in pCO_2 in the blood.

Alternatively, as a result of exposure to cold temperatures, CO_2 will become more soluble in water (Lumb, 2000). Therefore, as the CBT decreases, in order to maintain a homeostatic pCO_2 in the blood, a greater concentration of CO_2 will be required (Lumb, 2000). Finally, colder temperatures reduce the ability of water to ionize, causing an increase in the pH of the blood (Lumb, 2000). As a result of cold stress, both the pCO_2 and the pH of the blood would be expected to increase.

There has been limited research on the impacts of transportation and heat stress on the blood gases and blood pH of birds. However, results from existing studies are consistent. Studies have noted a significant decrease in the blood pCO_2 of birds exposed to temperatures above 30°C (Ait-Boulahsen et al., 1989; Koelkebeck and Odom, 1995; Sandercock et al., 2001). Koelkebeck and Odom (1995) noted a decreased in pCO_2 and bicarbonate, and an increase in the blood pH of laying hens after 60 minutes of exposure to high temperatures (38°C). Another study by Ait-Boulahsen et al. (1989) exposed broilers to 37°C and found the birds developed respiratory alkalosis with a sharp fall in blood pCO_2 and bicarbonate. Lastly, another study examined levels of blood gases and blood pH in broilers exposed to heat stress (32°C and 75%RH) for two hours (Sandercock et al., 2001). This study found that the birds exposed to heat stress resulted in a decrease in blood pCO_2 levels, and an increase in blood pH. While changes in levels of blood pCO_2 and blood pH due to heat stress have been documented, responses of blood gases and blood pH to cold stress have not been well studied. Since levels of blood gases and blood pH are

mainly only affected by increased respiration during heat exposure, changes in blood pH and blood gases may only be good indicators of heat stress in birds.

1.6 Live Shrink

Microclimate conditions during transport can affect live weight loss (Petracci et al., 2001; Khosravinia, 2017). Live shrink can be defined as the weight loss that occurs pre-processing and may be an inevitable consequence of transportation (NFACC, 2018). Though live shrink may not be a direct indicator of welfare, it can be indicative of the degree of energy expenditure of birds during transport (NFACC, 2018). Large losses in live weight may, therefore, suggest poor welfare, as this can indicate birds using high amounts of energy to respond to conditions during transport (NFACC, 2018). Live shrink during transportation can be caused by many factors which include feed restriction, dehydration, defecation, or through the mobilization of energy from fat and protein stores for thermoregulation and response to stressors during transport (Khosravinia, 2017).

Thermal stress is one of the most significant stressors for poultry during transport and can greatly influence live shrink (NFACC, 2018). Exposure to either heat stress or cold stress will cause birds to thermoregulate to maintain core temperature and physiological homeostasis (NFACC, 2018). Thermoregulation can be metabolically and physiologically demanding, therefore, birds must use energy in order to thermoregulate, and in extreme cases, considerable amounts of energy may be used (NFACC, 2018). To respond to stressors during transport, birds will initially use available glucose in the blood for energy (Sherwood et al., 2013). Once this energy is used, increased functioning of the hypothalamic-pituitary-adrenal (HPA) axis and corticosterone in the body will result in mobilization of glucose stores in the body which will be deposited in the blood to be used as energy (Sherwood et al., 2013). Thermoregulatory behaviours performed during heat and cold stress such as panting and shivering, respectively, are energy demanding, resulting in large amounts of energy mobilization, and a greater loss in live weight (NFACC, 2018). However, with high temperatures and high ambient humidity, birds must thermoregulate through panting, or evaporative heat loss, which can cause a higher weight loss in birds (Minka and Ayo, 2009). Depending on the environmental conditions, stressors and factors during transportation can be exacerbated by long transport durations leading to even greater increases in live shrink, which can suggest poor bird welfare.

With regards to cold stress, Dadgar et al. (2012b) found that broilers exposed to temperatures of -5ºC to -10ºC for three hours had the highest live shrink (3.4% and 3.5%, respectively), compared to broilers exposed to 22ºC for three hours (the lowest loss in live weight (1.7%)). The authors noted that the greater loss in live weight was a result of birds being feed restricted, having low energy reserves, and coping against cold conditions (Dadgar et al., 2012b). Furthermore, another study found a 4.20% loss in live weight from broilers exposed to cold conditions (-12.67°C, 59.53% RH) for three hours, while those exposed to neutral conditions (21.12°C, 22.60% RH) for three hours had a 1.36% loss in live weight (Dadgar et al., 2011). In the case of heat stress, Vermette et al. (2017) noted that turkey toms exposed to 35°C for three hours had a significantly higher live shrink compared to those exposed to 20°C for the same duration. These authors noted that this increased loss in live weight was likely a result of both dehydration and increased energy expenditure for thermoregulation against heat stress (Vermette et al., 2017). Increased transportation duration can also influence live shrink. Bianchi et al. (2005) transported broilers for various durations and found the highest live shrink in birds transported for over five hours (2.09%) compared to birds transported for three and a half hours or less (1.27%). However, in this study, transportation conditions were not mentioned, which may have influenced the loss in live weight (Bianchi et al., 2005). Unlike pullets, broilers are feed restricted before transportation which may lead to a greater loss in live weight during transportation. Nonetheless, thermal stress, long transportation durations, and potential interactions between the two factors can be significant stressors for birds, resulting in high energy demands and losses in body weight during transport.

1.7 Muscle Tissue Characteristics

1.7.1 Muscle Tissue pH

Though pullets do not provide a source of meat products for human consumption, muscle tissue characteristics such as pH can be a useful indicator of stress (Minka and Ayo, 2009). Both heat and cold stress can influence the ultimate pH of muscle through either glycogen build-up or depletion (Pearson and Young, 1989; Lyon and Buhr, 1999; Sandercock et al., 2001). Changes in muscle pH post-mortem are dependent on the level of glycogen in the muscle and the rate of anaerobic glycogen metabolism (Pearson and Young, 1989). After death, blood flow and oxygen delivery to the muscle ceases, but the muscle will continue to go through anaerobic glycolysis

producing ATP and pyruvate from the breakdown of glucose; pyruvate will then be converted into lactate by lactate dehydrogenase (Pearson and Young, 1989; Honikel, 2004). Additionally, stress pre-slaughter can cause muscles to go through anaerobic glycogen metabolism, causing a build-up of lactate in the muscle through anaerobic glycolysis (Honikel, 2004). A buildup of lactate will then cause the pH of the muscle to decrease (Pearson and Young, 1989; Honikel, 2004). Depending on the muscle, lactate production can cause the pH to fall from approximately 6.5 to 5.8 (Honikel, 2004). The amount of glycogen in the muscle post-mortem, as well as the rate of anaerobic glycolysis, can influence the resultant amount of lactate produced, which can thus affect the final pH of the muscle (Pearson and Young, 1989; Honikel, 2004). Muscles that have a high level of residual glycogen post-mortem will produce more lactate, causing a large drop in the muscle pH (Pearson and Young, 1989; Mahon, 1999). However, if birds experience high amounts of stress and exhaustion ante-mortem, this can lower the amount of glycogen in the muscle post-mortem, potentially resulting in an increase in the muscle pH (Lyon and Buhr, 1999; Mahon 1999).

Responding to the thermal stress experienced during transport may require birds to mobilize and utilize glycogen stores in the body to provide energy for the performance of various thermoregulatory behaviours and physiological processes to maintain homeostasis (Zhang et al., 2009). This can result in the depletion of glycogen in the muscle ante-mortem. The blood can remove the lactate that is produced in the muscle and carry it to the liver (Pearson and Young, 1989). With minimal residual glycogen in the muscle to go through post-mortem glycolysis, and with lactate being carried away by the blood ante-mortem, there may either be no change in the pH, or a slight increase in the pH post-mortem (Lyon and Buhr, 1999; Dadgar et al., 2011; Dadgar et al., 2012b).

The temperature of the muscle and the rate of anaerobic glycolysis can also affect the amount of lactate production and the final pH of the muscle (Lyon and Buhr, 1999). The temperature of the muscle has a significant effect on the rate of glycolysis, therefore, it can affect the rate of pH decline in the muscle (Lyon and Buhr, 1999). Muscles that have a higher temperature will have a faster rate of glycolysis and will produce lactate much faster (Lyon and Buhr, 1999). Therefore, muscles at a high temperature will have an accelerated decline in pH (Lyon and Buhr, 1999). On the other hand, cold temperatures tend to slow the rate of glycolysis and lactate production, causing a much smaller pH drop in the muscle (Lyon and Buhr, 1999).

Therefore, if birds are exposed to hot or cold temperature ante-mortem it can cause either an increase or decrease in the muscle temperature, respectively, which can affect the rate of glycolysis, and the rate of pH decline (Lyon and Buhr, 1999; Mahon, 1999).

Results from previous research have indicated that the muscle pH in birds can change as a result of exposure to either heat stress or cold stress. Dadgar et al. (2010) reported that birds exposed to 25°C for three hours had an average muscle pH of 5.84, which was lower compared to birds exposed to 15°C, 5°C, or -7°C for the same duration. Petracci et al. (2001) found that there was a trend (P=0.0718) towards a lower pH (5.86) in the muscle of broilers subjected to 34°C for 12 hours, compared to the control (29.5°C) birds (5.96); though this data trend follows the previously described study, care must be taken in the interpretation of this data, as the differences were not significant. With regards to cold stress, Dadgar et al. (2010) also noted a higher pH (5.98) in birds exposed to -7°C for three hours, compared to birds exposed to 5°C, 15°C, or 25°C for the same duration. A further study by Dadgar et al. (2011) exposed broilers to cold temperatures (-15°C) for three hours and found that, compared to the control group (21°C), the pH was higher for broilers exposed to cold stress (6.04 vs. 6.36). Therefore, post-mortem muscle pH can change depending on thermal conditions and stress experienced during transport. Taken together, changes in muscle pH may be a valuable measure of whether birds have experienced heat or cold stress during transportation.

Finally, no effect of transportation duration on the pH of muscle post-mortem has been observed in previous research. Kannan et al. (1997) and Zhang et al. (2009) exposed broilers to various transportation durations and lairage periods before processing and found no effect of either on the final muscle pH of broilers muscles. Results from these studies may indicate that the broilers had used their muscle glycogen stores during transportation, therefore, they did not have glycogen to convert to lactate post-mortem; however, this cannot be confirmed by the results presented in either of the studies mentioned above.

1.7.2 Drip Loss, Thaw Loss, and Cook Loss

The drip loss and cook loss of meat are attributes that may determine consumer acceptance of a product; however, they can also be used as indicators of thermal stress in birds. Drip loss is defined as being the loss in the weight of the muscle tissue caused by dripping of muscle tissue juices during thawing (Honikel and Hamm, 1994; Dadgar et al., 2012a). Cook loos is defined as the loss in muscle tissue weight as a result of cooking to a specified set point (75°C

internal temperature) (Honikel and Hamm, 1994; Dadgar et al., 2012a). Both drip loss and cook loss are measurements of the water-holding capacity of a muscle, which is defined as the muscle's ability to bind and retain water (Pearson and Young, 1989; Honikel and Hamm, 1994). In muscle, water can be bound and retained within the protein filaments in the muscle tissue, or it can be free, meaning that the water is available to be lost (Pearson and Young, 1989). The water-holding capacity of muscle is highly correlated with muscle pH, as the conformation of the proteins in the muscle and their ability to retain water will change with either a more acidic or more alkali pH (Pearson and Young, 1989; Sandercock et al., 2001). Therefore, the water-holding capacity of the muscle can change as a result of thermal stress and exhaustion post-slaughter (Pearson and Young, 1989). As pH in the muscle falls, the myosin and actomyosin filaments approach their isoelectric point (pH of approximately 5) (Pearson and Young, 1989). At this point, the amount of negatively and positively charged amino acid side chains become equal, and there is a high attraction between the protein filaments (Honikel, 2004). This causes the space between the filaments to decrease, and the water in this space moves to the sarcoplasmic space (Sandercock et al., 2001; Honikel, 2004). Water in the sarcoplasmic space is no longer bound and is now free water, therefore, the drip loss and cook loss will increase (Honikel, 2004). Though the same water-holding capacity mechanisms control both drip loss and cook loss, drip loss may only amount to approximately 3% of the weight loss of a muscle, whereas, in the same muscle, cook loss can amount to 25-35% (Honikel and Hamm, 1994). Thus, stressors prior to slaughter can potentially have a significant impact on the drip and cook loss of the muscle tissue.

On the other hand, stress and exhaustion ante-mortem, as well as cold muscle temperatures, may cause a reduction in the drip loss and cook loss by yielding a greater water-holding capacity post-mortem. As indicated in the previous section, exhaustion and colder muscle temperatures can cause the pH in the muscle to increase post-mortem (Pearson and Young, 1989; Lyon and Buhr, 1999). At a higher pH, the mechanisms described above can be counteracted, leading to more space between the protein filaments, thus more water is able to be bound in the filaments and remains in an immobilized form (Sandercock et al., 2001; Honikel, 2004; Dadgar et al., 2010). With more bound water, less water will be lost during thawing and cooking, resulting in a low drip loss and cook loss (Sandercock et al., 2001; Honikel, 2004; Dadgar et al., 2010). Therefore, chronic stress leading to exhaustion post-slaughter, as well as

cold stress, can lead to the muscle tissue having an increased water-holding capacity and a reduced drip loss and cook loss (Dadgar et al., 2010).

Many studies have examined changes in the drip loss and cook loss of muscles from birds exposed to hot environmental conditions pre-slaughter. A study by Dadgar et al. (2010) exposed broilers (unknown feed restriction) to various temperatures using a University of Saskatchewan test trailer, for approximately a three to four-hour duration, and found that exposure temperature had no effect on the drip loss of the breast; however, cook loss was highest when exposed to 25°C (14.98%) and 5°C (14.73%), and was lowest in birds exposed to 15°C (14.14%). Sandercock et al. (2001) exposed broilers (35 days of age) to heat stress (32°C with 75% relative humidity) for two hours and found a higher drip loss in the breast muscle of heat-exposed birds (3.7%) compared to the control birds (2.0%). Conversely, Holm and Fletcher (1997) observed different results in which birds exposed to 29°C had a lower cook loss (27.96%) compared to birds exposed to either 18°C (28.27%) or 7°C (27.96%).

Similarly, many studies have consistently shown an effect of cold stress on drip loss and cook loss. Breast muscle from broilers exposed to -15°C had the highest drip loss, whereas broilers exposed to -4°C had the lowest drip loss (Dadgar et al., 2011). Cook loss was highest in birds exposed to a neutral condition (21°C), and lowest in birds exposed to two different cold conditions (-15°C, and -13°C) (Dadgar et al., 2011). Similarly, Dadgar et al. (2012a) and Dadgar et al. (2012b) showed a significantly lower cook loss for broilers exposed to cold stress (below -9°C) for three hours. Not all results agree. Zhang et al. (2009) found no significant differences in the drip loss of breast muscle from broilers subjected to various transportation and lairage durations (45 minutes and three hours in a 2x2 factorial arrangement) (27°C and 76% RH environmental conditions inside crates). These results can indicate that by the end of the various transport and lairage durations, the birds were exhausted, therefore having very little glycogen store to alter the muscle pH and water-holding capacity post-mortem (Zhang et al., 2009). Studies examining muscle tissue quality parameters such as drip loss have primarily focused on broilers, as there is an economic value in quality broiler meat; however, muscle tissue quality parameters such as drip loss can be a useful indication of thermal stress in pullets but should be used in conjunction with other muscle tissue characteristic measures.

1.7.3 Muscle Tissue Colour

Meat colour is one of the most important quality attributes for the acceptance of poultry products (Pearson, 1994; Fletcher, 1999). Although pullets are not used for meat products, changes in pullet muscle tissue colour can be used to indicate stress in the birds. Thermal stress is a significant stressor during transportation, which could result in changes in the muscle tissue colour from birds post transportation. Muscle tissue colour can be categorized into the paleness (L*), redness (a*), and yellowness (b*) categories (Bianchi et al., 2006). Changes in muscle colour are highly correlated with muscle pH, with a high pH resulting in pale, soft, and exudative (PSE) muscle tissue, and a low pH resulting in dark, firm, and dry (DFD) muscle tissue (Fletcher, 1999). As the name suggests, PSE muscle tissue typically ranges from either pale grey to a white colour (high L*, and low a* and b* values), and this colour is caused by high amounts of anaerobic glycolysis and rapid muscle pH declines (Pearson, 1994; Bianchi et al., 2006; Dadgar et al., 2010). Rapid pH declines can have a prominent effect on the ultimate muscle tissue colour and can cause excessive denaturation of muscle proteins, resulting in the pale colour of PSE muscle tissue (Pearson, 1994). Additionally, a rapid decline in the muscle pH can result in precipitation of sarcoplasmic proteins, which mask the red colour of the muscle tissue, giving it a paler appearance (Person and Young, 1989).

On the other hand, DFD muscle tissue is usually characterized by a dark red to purple colour (low L*, and high a* and b* values), and is caused by glycogen depletion pre-slaughter which results in early-onset rigor and is typically associated with a higher pH (Fletcher, 1999; Dadgar et al., 2012a). An increase in pH can affect the muscle and produce a dark colour through two mechanisms (Pearson, 1994). First, at a high pH, mitochondria in the muscle will have a high oxygen consumption rate which remains over a long period of time (Pearson, 1994). Consequently, when the muscle is exposed to air, the oxymyoglobin layer on the surface will become thin, and the purple colour of the myoglobin will dominate (Pearson, 1994). Secondly, muscle tissue with a high pH has a high water binding capacity which causes greater translucence and less scattering of light, which allows for more absorption of light causing a darker appearance (Pearson, 1994). Therefore, muscle colour may be a useful indicator of stress in birds, as various stressors including heat or cold stress will cause changes in the pH of the muscle post-mortem, which will ultimately cause either PSE or DFD muscle tissue.

Results for changes in muscle tissue colour due to heat stress and cold stress have been variable. Petracci et al. (2001) found that broilers (45 days of age) exposed to 34°C for 12 hours had significantly lower a* and b* values, compared to broilers exposed to cool (25°C) or control (29.5°C) conditions, for the same duration. Dadgar et al. (2010) reported that broilers exposed to heat stress (25°C) for three hours had the highest L* values and lowest a* values compared to broilers exposed to neutral (15°C) and cold temperatures (5°C, and -7°C). With regards to cold stress, several studies have shown that when broilers are exposed to temperatures below 0°C, the resulting breast and/or thigh muscle tissue is darker (lower L*) and redder (higher a*), compared to breast and/or thigh muscle tissue from broilers exposed to temperatures above 20°C (Dadgar et al., 2011; Dadgar et al., 2012a). Bianchi et al. (2006) found that the breast muscle tissue of broilers exposed to temperatures below 12°C was darker (lower L*) compared to those exposed to temperatures between 12 to 18°C. Sandercock et al. (2001) found no effect of heat stress on any breast muscle colour parameters, which may have signalled fatigue in birds pre-slaughter. Therefore, differences in muscle tissue colour post-slaughter can be used to quantify either heat stress, cold stress, or exhaustion in pullets post-slaughter. Most studies have looked at broiler muscle tissue colour, as there can be an economic consequence to discolouration post-slaughter.

1.8 Conclusions

Transportation is a key component to the poultry industry, however, environmental conditions during transport, and journey lengths may present a significant welfare concern. Hot temperatures, with varying humidity, as well as cold temperatures, can be significant stressors for birds during transport and can cause increased incidences of mortality. Furthermore, long transportation durations can exacerbate poor conditions present during transport, which can be an additional welfare concern. It is essential to understand the behavioural changes and physiological impacts on birds during transport to determine if transportation conditions present a welfare issue. Previous research has shown that thermal stress, as well as longer transportation durations, can result in behavioural changes, and negative impacts on bird's physiology. The majority of previous research has focused on broilers, and the effects on pullets are still not well understood. Additionally, little is known regarding the impact of thermal stress and increased transportation duration in conjunction. The data from this study will aid in understanding the overall impact of both heat stress, cold stress, and increased transportation durations, on the behaviour, physiology, and muscle tissue characteristics in pullets.

1.9 Objectives

The overall objective for this study was to evaluate and assess the stress responses exhibited by white-feathered commercial layer pullets when exposed to hot and neutral temperatures, with a low and high humidity, as well as to a cold temperature, for a four or eight-hour duration.

To accomplish this objective, several other objectives were included:

1. to evaluate behaviours performed by pullets when exposed to heat stress or cold stress
2. to evaluate and quantify the physiological stress responses exhibited by pullets in response to heat and cold stress and
3. to quantify and compare the changes in muscle tissue characteristics due to heat stress and cold stress in pullets

1.10 Hypotheses

The overall hypothesis for this study is that exposure to hot temperatures, with a high or low humidity, or to a cold temperature, for different durations will negatively affect the welfare of pullets. As a result of exposure to either heat or cold stress, pullets will implement behavioural and physiological mechanisms to thermoregulate in response to the conditions. However, these mechanisms may be inadequate in maintaining homeostasis, resulting in negative physiological changes, and potentially mortality. Additionally, an increased exposure duration will exacerbate the effects resulting from exposure to either hot or cold conditions.

The specific hypotheses for this study are as follows:

Behaviour:

1. Active and thermoregulatory behaviours performed by pullets will increase due to the need to dissipate heat during heat stress or conserve heat during cold stress.

Physiology:

1. Core body temperature will increase due to exposure to a hot temperature, regardless of humidity, while core body temperatures will decrease due to exposure to a cold temperature.

2. Due to exposure to hot temperatures, regardless of humidity, vasodilation of the blood vessels to the feet will occur as a mechanism to dissipate heat, resulting in a higher skin temperature. Due to cold exposure, vasoconstriction of the blood vessels to the extremities will occur to conserve heat for the core body, resulting in a colder skin temperature.
3. As a response to both heat and cold stress, blood glucose will initially decrease as it is being used for thermoregulation. As glucose stores are mobilized to further respond to the environmental conditions, blood glucose levels will increase.
4. Plasma electrolytes (sodium) will decrease in birds exposed to hot or cold treatments as a result of defecation and dehydration during exposure
5. Base excess in the extracellular fluid will increase as a result of heat exposure, with a high or low humidity, due to an increase in panting behaviour causing a respiratory alkalosis.
6. Pullet hematocrit and hemoglobin will increase due to dehydration from simulated transportation resulting less fluid in the blood.
7. The partial pressure of carbon dioxide, and the total carbon dioxide in the blood will decrease due to panting in response to exposure to high temperatures, with a high or low relative humidity, which will then result in an increase in the blood pH and a respiratory alkalosis
8. The partial pressure of oxygen and the soluble oxygen in the blood will not change as a result of heat or cold stress, as oxygen levels in the blood cannot be altered through changes in behaviour resulting from thermal stress.
9. Bicarbonate in the blood will decrease in response to heat stress, as panting will cause a decrease in the partial pressure of carbon dioxide in the blood, and bicarbonate levels will adjust accordingly.
10. There will be an increase in the heterophil to lymphocyte ratio as a response to chronic stress through an increased production of heterophils, and decreased production of lymphocytes in the body.

Muscle Tissue Quality:

1. Pullets will experience a loss in body weight post-treatment due to gut emptying, dehydration, and expending energy in order to thermoregulate during exposure to both hot and cold treatment conditions.
2. Due to exposure to hot treatments, at both a high and low humidity, the rate of post-mortem glycolytic metabolism in muscle will increase, causing glycogen and glucose to be converted to lactate, which will decrease the pH of the muscle. Due to exhaustion and exposure to the cold treatment, muscles will be depleted of glycogen, thus there will be a slight increase in the muscle pH.
3. The drip loss, thaw loss, and cook loss from the breast and thigh muscles will be higher as a result of exposure to both hot treatments, due to the decreased pH of the muscle causing a decrease in the muscles ability to retain water. Drip, thaw, and cook loss will be lower as a result of cold exposure, as an increase in pH will result in the muscle having a higher water-holding capacity.
4. As a result of increase post-mortem glycogen metabolism and acidification of muscle after exposure to both hot treatments, breast and thigh muscle tissue from birds will become pale, soft, and exudative. As a result of less post-mortem glycogen metabolism in the muscle of pullets exposed to the cold treatment, the breast and thigh muscles will become dark, firm, and dry (DFD).

2.0 Chapter 2: The effects of simulated transport conditions on the core body and extremity temperature, behaviour, and blood physiology of white-feathered layer pullets

The objectives of this study were to examine and assess how exposure to various temperature and humidity combinations for different durations affects the welfare of white-feathered egg production pullets. Chapter 2 focuses on the effects on pullet core body temperature, behaviour and physiological stress responses. Physiological stress responses include foot temperatures, heterophil to lymphocyte ratio, core body temperature, blood gases, blood electrolytes, blood pH, blood glucose, hemoglobin, and hematocrit.

Many individuals were involved with the current study. My role, as first author, was in the collections, analyses, and presentation of the data, and in preparing the following thesis. Kailyn Beaulac was essential in the preparation, and scheduling of this study. Dr. Trever Crowe and, my advisor, Dr. Karen Schwean-Lardner obtained the funding for this research, planned the experimental design, offered valuable knowledge, and served as editors and reviewers for this thesis. Finally, further review and editing were conducted by my advisory committee members, Dr. Denise Beaulieu, and Dr. Jennifer Brown, and my graduate chair, Dr. Fiona Buchanan.

2.1 Abstract

The objective was to determine the effects of various temperature (T)/relative humidity (RH) combinations and exposure durations (D) on the physiology and behaviour of pullets during simulated transport. The experiment was a 5x2 factorial arrangement of T/RH combinations, and exposure D (3 replications). Pullets (n=240, 8 per T/RH and D) were randomly exposed to one of five combinations (21°C with 30%RH (21/30), 21°C with 80%RH (21/80), 30°C with 30%RH (30/30), 30°C with 80%RH (30/80), and -15°C with uncontrolled RH (-15)), for a four or eight hour D, in environmental chambers. Pre-treatment, birds were orally administered a data logger to record core body temperature (CBT). Blood was collected pre- and post-exposure from five birds/T/RH and D and analyzed for heterophil to lymphocyte (H/L) ratio, and three samples were analyzed for partial pressure of CO_2 (pCO_2), total CO_2 (tCO_2), bicarbonate, and glucose. Foot T were recorded for pullets pre- and post-exposure. Behaviour during exposure was video recorded. Following exposure, birds were slaughtered, and data loggers were retrieved. In a randomized complete block design, data were analyzed via Proc Mixed (SAS 9.4). Differences were determined as $P \leq 0.05$. Final glucose was higher in birds exposed to 30/80 compared to 21/80 and 21/30. Final foot T were highest in birds exposed to 30/80, compared to those exposed to the cold and neutral treatments, while foot T were lowest in birds exposed to -15 compared to the other treatments. Panting and surveying occurred more in 30/30 and 30/80 pullets, while 30/80 birds were more active. Pullets exposed to 30/30 pecked conspecifics more frequently. Preening occurred more in 30/30 and 21/80. Pullets in -15 and 21/30 remained motionless for longer, compared to 30/30 and 30/80. The ΔCBT was higher in the eight hour D. With increasing D, ΔpCO_2, ΔtCO_2, and Δbicarbonate decreased. Activity was higher in the four hour, than the eight hour, D. These data indicate that as a response to thermal stress, birds mobilized more energy, and performed more thermoregulatory behaviours. Foot T increased or decreased due to hot or cold exposure. A longer D spent hyperventilating resulted in a shift towards a respiratory alkalosis.

Keywords: cold stress, heat stress, thermoregulation, hematology, welfare

2.2 Introduction

Transportation is a fundamental component of the poultry industry; however, it can also result in animal welfare concerns (Schwartzkopf-Genswein et al., 2012). Poultry transportation can encompass many steps, including catching, loading, transportation, and unloading (Schwartzkopf-Genswein et al., 2012). Unlike broilers and laying hens, pullets are typically not feed withdrawn before transportation (Schwartzkopf-Genswein et al., 2012). Rather, they are usually transported from a rearing barn to a laying barn at approximately 17 weeks of age, and this transportation can be over either a very short or very long distance (Schwartzkopf-Genswein et al., 2012; NFACC, 2016).

Transportation can subject birds to a variety of stressors, including feed withdrawal, handling, social disruptions, vibrations, and noise (Mitchell and Kettlewell, 1998; Mitchell and Kettlewell, 2009). However, the most significant stressor during transportation may be the microclimate environment (constituting both temperature (T) and relative humidity (RH)) within the trailer (Mitchell and Kettlewell, 1998; Mitchell and Kettlewell, 2009). Currently, the most common types of transportation trucks in Canada rely on passive ventilation, which can be inadequate at circulating both heat and moisture within the trailer (Knezacek, 2005). This can create large T and RH gradients within the trailer, particularly when tarpaulins are used (Knezacek, 2005). These T and RH gradients can create poor environments for birds during transport, which can be a welfare concern, and long transportation durations may exacerbate stressors present during transport (Mitchell and Kettlewell, 1998; Mitchell and Kettlewell, 2009). High and low T within the trailer can influence the core body temperature (CBT) of birds during transport, which may result in either hyperthermia or hypothermia, which is a welfare concern (Mount, 1979; Mitchell and Kettlewell, 2009).

Chickens are homeotherms, where the CBT of inactive birds is approximately 41-42ºC (Mount, 1979). As a first response to cold stress, birds will change their behaviour in order to conserve heat (Mount, 1979). They will attempt to minimize radiation to the environment and maximize conduction between conspecifics (Mount, 1979; Strawford et al., 2011). To do this, birds will burrow under one another and huddle to minimize the amount of body surface area exposed to the environment (Mount, 1979; Strawford et al., 2011). Feathers aid in providing insulation against exposure to cold T, and through ptiloerection, air can be trapped between the

feather fibres to further aid in insulation (Mount, 1979; Strawford et al., 2011). To further conserve heat for the core body, blood vessels to the extremities (feet and combs) will vasoconstrict in order to restrict blood flow (Midtgard et al., 1989; Wellehan, 2014). However, if ambient T become too cold, the extremities can be susceptible to frostbite, and hypothermia may occur (Mount, 1979; Wellehan, 2014). Previous studies have consistently found that the CBT of broilers will decrease when exposed to T below -5ºC for three hours (Dadgar et al., 2011; Dadgar et al., 2012b). The lower critical limit for adult birds has been noted to be approximately 18-22ºC (Mount, 1979). If thermoregulatory mechanisms against cold stress are unable to maintain the bird's CBT, their welfare can be negatively impacted, and mortality may occur.

When exposed to hot T, birds may implement non-evaporative and evaporative heat-loss mechanisms to dissipate heat from the core body (Mount, 1979). Radiation and convection are the main non-evaporative mechanisms birds use to dissipate heat (Mount, 1979). When exposed to high T, blood vessels to the extremities will vasodilate, causing an increased blood flow, which can aid in dissipating heat through convection and radiation through the bare skin (Whittow et al., 1964; Richards, 1971; Midtgard et al., 1989). In order to ensure effective heat dissipation through radiation and convection, birds will move away from conspecifics and maximize space usage within the crate to ensure that the maximum body surface area is exposed to the environment (Mount, 1979; Weeks et al., 1997). When non-evaporative heat loss methods fail to maintain the bird's body T, they will implement evaporative heat loss mechanisms (panting) to dissipate heat (Whittow et al., 1964; Mount, 1979). However, when the RH in the environment is high, panting can become ineffective at dissipating heat from the core body (Whittow et al., 1964; Richards, 1971). It has been shown in previous research that the CBT of broilers will significantly increase as a result of exposure to T above 30ºC for two hours (Sandercock et al., 2001; Menten et al., 2006). The upper critical T for adult birds is approximately 47ºC, and when the implementation of behavioural thermoregulatory mechanisms is inadequate at maintaining body T, the birds may be at risk for hyperthermia (Whittow et al., 1964; Richards, 1971; Mount, 1979). However, when behavioural mechanisms become inadequate to maintain the bird's body T, other physiological mechanisms can be employed.

Thermal stress can also alter the blood physiology of birds. Measuring various blood physiology parameters such as blood glucose, acid-base balance parameters (blood pH, base

excess in the extracellular fluid (BEecf), and bicarbonate), levels of blood gases (partial pressure of carbon dioxide (pCO_2), total carbon dioxide (tCO_2), partial pressure of oxygen (pO_2), and soluble oxygen (sO_2)), dehydration markers (hematocrit, hemoglobin, and sodium (Na)), and the heterophil to lymphocyte (H/L) ratio, can give an indication of the degree of stress experienced by birds during transportation. Due to dehydration, the amount of fluid in the blood will decrease, increasing both the hematocrit and hemoglobin values (Thomas et al., 2008; Bergoug et al., 2013). However, it has also been hypothesized by previous research that the hematocrit and hemoglobin values may decrease through hemodilution, which is an adaptive response to evaporative heat loss where water is lost through the extracellular fluid rather than through the plasma (Borges et al., 2004; Scanes, 2016). In addition, the body will lose water (via dehydration, and defecation), and electrolytes (through mainly defecation) resulting in low blood sodium levels and hyponatremia (Thomas et al., 2008). Furthermore, as a result of stress during transportation, corticosterone in the body will increase, causing glucose to be mobilized from the fat stores in the body through glycogenolysis, and from protein stores through gluconeogenesis (Zhang et al., 2009; Sherwood et al., 2013). This glucose will then be deposited into the blood and can be used as energy to respond to stressors and thermoregulate against heat or cold stress. As a result of evaporative heat loss during heat exposure, blood gases and the acid-base balance will change, causing a shift towards a respiratory alkalosis (Koelkebeck and Odom, 1995; Lumb, 2000). Hyperventilation during heat exposure will increase the elimination of CO_2, resulting in a decrease in the pCO_2 and tCO_2 in the blood, causing an increase in the blood pH (Ahmad and Sarwar, 2006; Sherwood et al., 2013). Finally, the H/L ratio has been shown to be a reliable indicator of chronic stress in poultry (Felver-Gant et al., 2012). Stress will cause corticosterone to increase which will, in turn, resulting in either an increase or decrease in the number of heterophils and/or lymphocytes, ultimately causing an increase in the H/L ratio (Blecha, 2000; Elsayed, 2014). Therefore, changes in the blood physiology of pullets during transport may indicate that they were subjected to thermal stressors during transport and thus may indicate poor welfare.

Most studies regarding poultry transportation have focused on broilers, and there is very little known regarding the transportation of pullets. Therefore, the objective of this chapter was to quantify how exposure to hot or neutral T, at a high or low humidity, as well as a cold T, affects pullet CBT, behaviour, feet T, blood physiology, and H/L ratio in an attempt to

understand the impact on bird welfare. Parameters assessed will include CBT, percentage of time performing various behaviours, foot T, blood glucose, hemoglobin levels, blood Na, the hematocrit levels, blood gas levels (pCO_2, tCO_2, pO_2, and sO_2), acid-base balance (blood pH, BEecf, and bicarbonate), and the H/L ratio. It was hypothesized that as a result of heat stress, the CBT would rise, and foot T would also increase as vasodilation to the extremities occurs. During cold exposure, pullet CBT will decrease, as well, foot T will decrease due to vasoconstriction of the blood vessels to the extremities. It was also hypothesized that in response to thermal stress, pullets would spend more time performing thermoregulatory behaviours in order to cope with the environmental conditions, and more energy will be mobilized in order to support thermoregulation. Due to panting during heat stress, blood physiology will change in association with the development of a respiratory alkalosis. A high humidity will exacerbate responses observed with exposure to high temperatures, as a result evaporative heat loss becoming ineffective. Dehydration and defecation during transportation will result in low blood Na levels, as well as higher hematocrit and hemoglobin levels. Additionally, the H/L ratio of pullets during simulated transport would increase due to chronic exposure to stressors. Lastly, due to an increased transportation duration, stress responses exhibited by pullets would be exacerbated.

2.3 Materials and Methods

The experimental protocol for this research was approved by the University of Saskatchewan Animal Care Committee and was performed under the guidelines of the Canadian Council of Animal Care (1993, 2009).

2.3.1 Experimental Design

To test the effects of various T and RH combinations on the welfare of commercial layer pullets, five exposure treatments were used. These treatments included 30°C with 80% RH (30/80), 30°C with 30% RH (30/30), 21°C with 80% RH (21/80), 21°C with 30% RH (21/30), and -15°C with uncontrolled humidity (-15). As well, there were two-time durations (D) tested for each treatment, four or eight hours of exposure. A total of 240 white-feathered commercial layer pullets were used in this study. Pullets were 18-19 weeks of age. The study consisted of three replicates, with 80 pullets per replicate (bird flock). Two transport crates were used per treatment per replicate, with one crate per time duration for each replicated run. These treatments were performed with two treatments per night, for a total of three nights (Figure 2.1).

Crate Rotation Schedule					
		Chamber One		Chamber Two	
Day	Time	In	Out	Crates In	Crate Out
1	5pm	C1-4h C2-8h			
	7pm			C3-4h C4-8h	
	9pm		C1		
	11pm				C3
	1am		C2		
	3am				C4
		Chamber One		Chamber Two	
2	5pm	C1-4h C2-8h			
	7pm			C3-4h C4-8h	
	9pm		C1		
	11pm				C3
	1am		C2		
	3am				C4
		Chamber One			
3	5pm	C1-4h C2-8h			
	9pm		C1		
	1am		C2		

Figure 2. 1. Crate rotation schedule for each treatment during the study. Treatments were randomly allocated to each day for each replication according to the relative humidity (RH) capacity of each chamber (chamber one capacity 100%RH, chamber two capacity 60%RH).

C1= Crate 1, C2= Crate 2, C3= Crate 3, C4= Crate

Using two environmental simulation chambers (College of Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada) (Figure 2.2, 2.1 m x 3.4 m), the five treatment conditions were created. The RH of one chamber was controlled by a steam generator (Nortec, H.V.A.C Sales (1995) LTD), which was controlled by a computer software system (LabVIEW). The humidity in the second chamber was controlled by either one or two humidifiers (Essick Air Aircare Products Evaporative Humidifier), or a dehumidifier (NOMA Dehumidifier), as necessary. The systems controlling the T and RH within the chambers were turned on and given approximately two hours to stabilize to their respective conditions before pullets were transported to the chambers. Prior to the pre-treatment procedures, two identical crates were placed on top of wooden tables inside each of the chambers (Figure 2.3). The crates were labelled with the respective treatment and time duration and were equipped with a T and RH data logger (Hygrocon iButton DS19223, Maxim Integrated Products) at bird level. Removable lids constructed of chicken wire and a wood frame were placed on top of the crates (Figure 2.2). A thermocouple attached to a multimeter (Omega HH509, Omega Engineering Inc., Laval, Quebec, Canada) was attached to the side of the chamber, at bird level, to monitor chamber T in real time. A humidity sensor, which was interfaced to the same computer system as used for the steam generator, was also attached to the side of the chamber at bird level and used to monitor the humidity of the chambers in real time. Two infrared camera systems (Panasonic WV-CF224FX; Panasonic Corporation of North America, One Panasonic Way 7D-4, Secaucus, NJ, USA) were placed above the wooden table inside each chamber, with one camera observing one crate (Figure 2.2). Lights within the chambers were turned off prior to commencing the pre-treatment procedures.

2.3.2 Birds and Housing

Pullets were purchased from commercial farms within a 250-kilometer radius of Saskatoon, Saskatchewan, and transported to a livestock facility at the University of Saskatchewan, where they were housed for up to six days. Birds were transported in one trip using an enclosed van. Information on farm of origin, bird strain, and age are presented in Table 2.1. Pullets were housed in one floor pen (Figure 2.4, 3.89 x 3.00 m) with straw litter and given a three to five day adaptation period before being exposed to chamber conditions. Between each flock, the pens were cleaned out, and new straw was placed. Feed was acquired from the producers at bird pick up and was provided via three tube feeders (38 cm diameter).

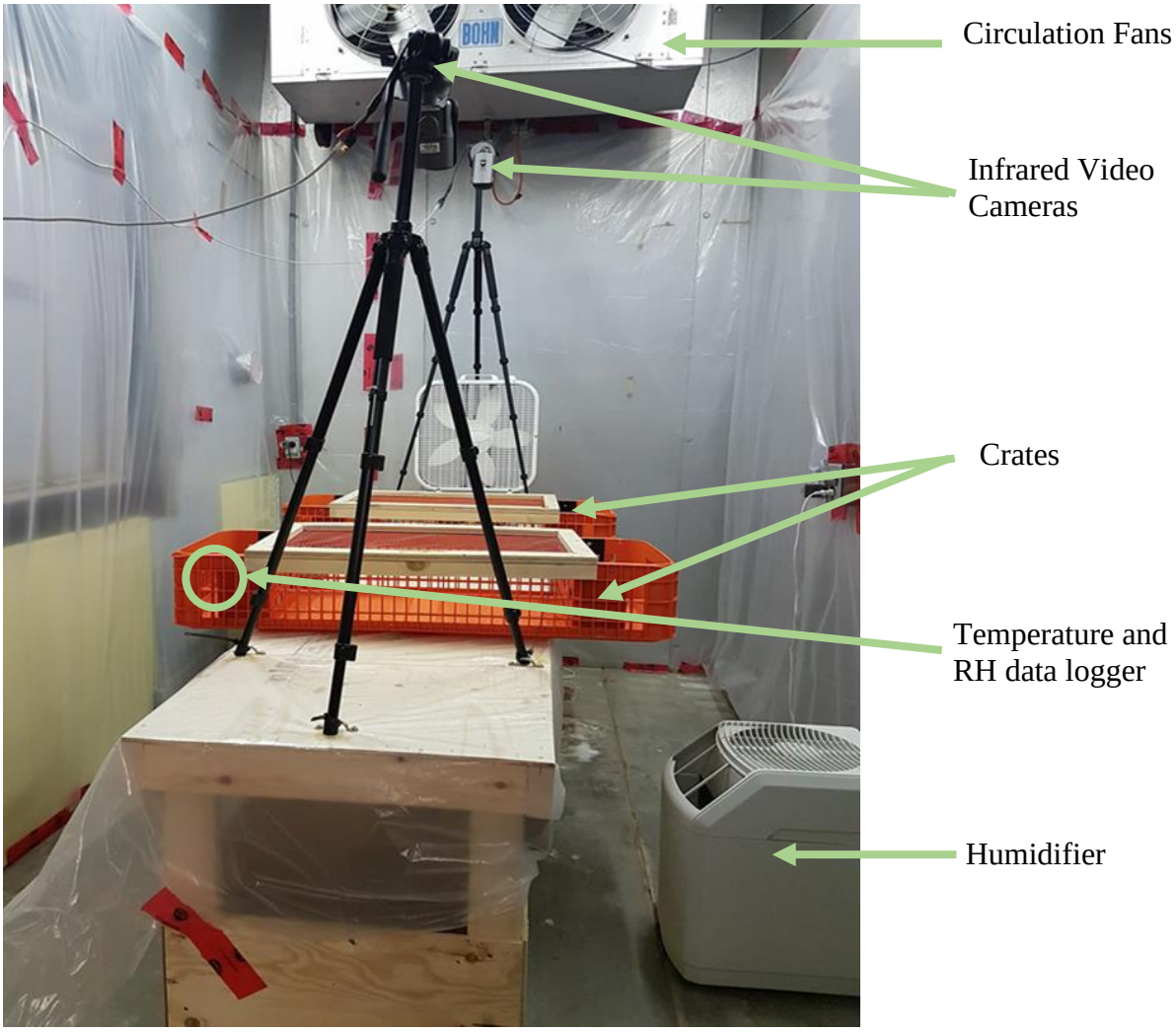

Figure 2. 2. Image of the inside of the environmental simulation chamber.

Figure 2. 3. Visual of the crates used to contain the pullets. One crate was used per time D (eight pullets per crate) for each T/RH combination and D replicate.

Table 2. 1. Summary of particulars associated with each replication of the current study.

Run	Farm	Strain	Age
Replicate 1 – March 2, 2018 – March 9, 2018	Pennant Farming Co.	Lohmann White	19 weeks
Replicate 2 – May 25, 2018 – June 1, 2018	Clear Springs Colony	Dekalb	19 weeks
Replicate 3 - September 7, 2018 – September 14, 2018	University of Saskatchewan Poultry Research and Teaching Unit	Lohmann White	18 weeks

Figure 2. 4. Image of the pen within the livestock housing facility used to house the pullets during the adaptation period. Only one pen was used.

Water was provided with two bell drinkers (36 cm diameter). Both feed and water were provided ad libitum. Information, including the lighting program and barn set T was obtained from the producer from the farm of origin and was implemented inside the livestock facility. Birds were checked twice daily with feeders filled as necessary. Pullets were not feed restricted before treatment exposure. No mortality occurred during the acclimation periods.

2.3.3 Pre-Treatment Procedure

On the day of collections, eight pullets were randomly assigned to one of the five T/RH conditions for either the four or eight hour D. During the pre-treatment procedures, these 16 pullets were individually prepared by administering two identifying wing bands to each bird, and administering a miniature data logger (DS1922L, Maxim iButton) orally by manipulating it into the crop. The miniature data loggers took approximately 15 minutes to equilibrate inside the crop of the bird (based on a previous pilot study). All data loggers from each replication were administered approximately ≥60 minutes prior to placement inside the chambers. Four pullets (two per crate) from the two extreme treatments (30/80 and -15) were administered a transponder (IPTT-300, Bio Medic Data Systems, Delaware, US), which was injected subcutaneously along the body, beneath the right wing, and were used to monitor instantaneous surface body T during exposure as an indicator for determination of humane endpoint for pullets possibly under distress (see section 2.3.5). Birds injected with transponders were identified by colouring their wings and backs with a livestock marker. Blood samples were taken from five birds per crate (ten birds per T/RH). These birds were not administered a transponder. After being prepared for the experiment, the pullets were placed into one of two crates (stocking density 45.5 kg/m^2) that was labelled with a respective time D for the treatment. Once all pullets for a T/RH combination across both time D were prepared and crated, the pullets remained in lairage for 15 minutes to obtain baseline CBT measurements. Baseline levels were obtained instead of calibrating data loggers, reading offsets were assumed to be constant over the operating range, therefore, delta (Δ) CBT values will be immune to various measurement offsets from the different data loggers and confidence can be taken from the Δ values. The pre-treatment procedures were conducted for all five T/RH combinations, and each D and the corresponding pullets were put into identical crates. After the lairage period, the crates containing the pullets were transported one and a half kilometres to the building containing the environmental simulation chambers (College of Engineering, University of Saskatchewan, Saskatoon, Saskatchewan). Pullet were transported in

one trip using an enclosed van. Once pullets arrived at the building, they were then transported to the room containing the environmental simulation chambers.

2.3.4 Treatment Procedure

After the pullets arrived at the room containing the environmental simulation chambers, foot T were recorded for each bird. Birds were then placed into the appropriate crate inside the chamber with the respective T/RH condition and for the predetermined D. Lights within the chambers remained off during the exposure period. Video cameras were turned on prior to pullets being placed inside the chambers. The behavioural activity was recorded to video for the entire four or eight hour D, depending on the D to which the crate was assigned. The crates were placed in and taken out of the chambers according to the schedule presented in Figure 2.1. Within the two extreme treatments (30/80 and -15) transponders were checked using a wand (BMDS IPTT DAD-8007-IUS) at intervals of 30 minutes during exposure to monitor the surface body T of the pullets. In all other treatments, every 30 minutes, birds were disturbed in a similar manner (sham treatment) by placing a hand on each bird in the crate and gently agitating the bird. Exposure conditions (T and RH) within the chambers were monitored and recorded in real time every 30 minutes using the thermocouple and RH sensor. The set points for T and RH were adjusted as necessary in order to maintain exposure conditions within the chambers. The air velocity was recorded using an anemometer inside the chamber at bird level at two different time points during each T/RH exposure, and ranges were 0 to 1.4 m/s in chamber #1, and 0.2 to 1.6 m/s in chamber #2. Once the crate was removed from the chamber, the respective behaviour video was downloaded and archived (Seagate Expansion Portable Drive, Model SRD0NF1). The crate containing the birds was then taken to a separate room for further bird analyses and slaughter.

2.3.5 Humane Intervention

In order to reduce birds' suffering, several humane intervention points were identified prior to testing and implemented while birds were subjected to the experiment. Pullets displaying behaviours associated with heat stress (panting, or increased activity) or cold stress (shivering, ptiloerection, or torpor) were not removed from the trials, as the objective was to observe and understand these behaviours and how they are influenced by the environmental conditions in this study. While transponder readings were recorded, birds within both crates were being monitored

by trained personnel for signs of birds appearing incapable of coping with the environmental conditions. Birds displaying distress behaviours (excessive panting or shivering, or torpor) and deemed unable to cope with the environmental conditions, as determined by the trained personnel, were removed from the study. If transponder readings exceeded 43°C, birds were monitored every 15 minutes for indicators of distress (outlined above). Additionally, if transponder readings were below 37°C birds were monitored every 15 minutes for signs of distress (outlined above). The end of this experiment was determined to be the removal from the environmental chambers after exposure. No birds were removed from the study as a result of humane intervention

2.3.6 Post-Treatment Procedure

At the end of the exposure period within the test chamber, the T and RH data logger was removed from the crate, and the data from the logger was downloaded onto a computer for further analyses. A final blood sample was taken from the same five pullets per crate (ten birds per treatment) as during the pre-treatment procedure. For the extreme treatments only (30/80 and -15), birds with identifying coloured wings (transponder birds) were euthanized via cervical dislocation by a trained individual. The transponders and miniature data loggers were then removed, and the carcasses were placed in a container for proper disposal. Once all final blood samples were collected, all remaining pullets were shackled and euthanized via stunning with an electric knife (VS200, Midwest Processing Systems, Minneapolis, MN, USA) at power level 6 (circa 0.16 amps, 60 Hz AC) for 30 seconds, or until the wing drop response and absence of the nictitating reflex was noted (Henrikson et al., 2018). Immediately afterwards, the pullets were exsanguinated by severing the jugular vein. Carcasses previously blood sampled were scalded (65-68°C) using a commercial scalder (Chicken Dipper Scalding Tank DUX, 120 volts), plucked mechanically using a commercial plucker (Featherman Feather Picker K7080, 10.8 Amps), and eviscerated. Carcasses then went on for further muscle tissue characteristic analyses (see Chapter 3).

2.3.7 Data Collection

Experimental collection procedures and data measurements collected are outlined in Figure 2.5. Within Figure 2.5, the colour of the procedure conducted coincides with that of the measurement taken for each time period. Procedures were performed as outlined for all three replicates. Once stopped, archived behaviour video was analyzed at a later date.

Core Body Temperature: Miniature data loggers recorded crop or gizzard T as an estimate for the CBT. Data loggers recorded T every 60 seconds while inside the crop or gizzard of the bird for the entirety of the respective D. Baseline values for each replication were obtained as follows: for the first replication baseline values were taken from minute five to minute ten of chamber entry. Between these two time points, pullets would have been placed inside the chambers, the miniature data loggers would have equilibrated to the pullets' temperature, and the length of time within the chamber conditions would not have been enough to influence the T of the pullets or the miniature data loggers. For replications two and three, baseline values were obtained during the 15-minute lairage period prior to chamber exposure. Differences in (Δ) CBT were calculated per pullet by subtracting the mean baseline CBT from the mean CBT during exposure. The miniature data loggers from all pullets were retrieved during evisceration. Once they were removed the data were downloaded using computer software (1-Wire) and were saved for further analyses.

Blood Measurements and Blood Smear Preparation: Blood samples were taken by drawing blood from the brachial wing vein using an Ethylenediamine Dipotassium Tetraacetic Acid (EDTA) anti-coagulation tube and vacutainer (BD Vacutainer). Three blood samples (six per T/RH and D) were tested via an iSTAT machine for various blood physiology parameters (Abaxis Vetscan i-STAT 1). Blood analyses were conducted by drawing blood from the EDTA tube using a plastic transfer pipette, placing two to three drops of blood into an iSTAT cartridge (I-STAT CG8+ Vet Scan) and placing the cartridge in the iSTAT machine. Analyses were conducted onsite on both pre and post-treatment blood samples for determination of levels of the blood gases (pO_2, sO_2, pCO_2, and tCO_2), glucose, sodium, bicarbonate, hemoglobin, hematocrit, the BEecf, and the blood pH. The same procedures were done for both the initial and final blood samples.

Time	Procedure	Measurement Taken
0:00h All pullets	Administered miniature data logger Blood samples taken Wing banded Crated (45.5 kg/m^2)	Initial blood parameters Initial H/L ratio CBT recording started
1:15h All pullets	Lairage period	Baseline CBT
1:45h All pullets	Toe temperatures recorded	Initial foot temperature
2:00h	Chamber entry (both durations)	Behaviour recording started
6:00h	4 hour crate removed	Behaviour recording stopped
10:00h	8 hour crate removed	Behaviour recording stopped
Pullets in both the 4- and 8-hour duration went through the procedures outlined below		
6:00h (4 hour) 10:00h (8 hour)	Toe temperatures recorded	Final foot temperature
6:15h (4 hour) 10:15h (8 hour)	Blood samples taken Stunning Exsanguination Scalding Plucking Evisceration	Final blood parameters Final H/L ratio Miniature data loggers removed

Figure 2. 5. Exerimental procedures and measurements recorded (modified from Henrikson, 2017)

H/L ratio = Heterophil to lymphocyte ratio
CBT = Core body temperature

Five blood samples (ten per T/RH and D), were used for H/L analyses. Samples were taken to a separate lab for preparation of blood smears. Smears for H/L ratio analyses were prepared according to the two-slide wedge method, wherein a small drop of blood was transferred from the sample tube via a stir stick and manually smeared. After drying, smears were stained with Ricca Wright-Giemsa stain and Giordano buffer according to the manufacturer-supplied procedure (Ricca Chemical Company, 2005) and stored in slide boxes. Heterophil to lymphocyte ratios were determined by viewing the slides under an oil immersion lens (1000x magnification). Heterophils and lymphocytes were identified and counted until a total of 100 combined heterophils and lymphocytes were reached, and the number of the former was divided by the latter to produce the heterophil to lymphocyte ratio. Procedures were conducted on both the initial and the final blood samples.

Foot Temperatures: Initial and final foot T were obtained from all pullets from all treatments using a thermocouple attached to a multimeter (Omega HH509, Omega Engineering Inc., Laval, Quebec, Canada), and a plastic clip to ensure the thermocouple remained in place. Foot T were taken by placing the thermocouple and clip on the middle toe of the right foot. Due to human error, foot T for all treatment conditions were not obtained for all replications, therefore, measurements were obtained as follows: for the first replication foot T were only obtained on pullets exposed to the 30/80 and -15 treatments. However, in the 30/80 four hour duration final foot T were not obtained, therefore the change in foot T for this treatment could not be calculated. For the second replication foot T were taken for all pullets in all treatments, although final foot T were not taken in the 30/30 four hour treatment, thus the change in foot T for this treatment could not be obtained. Lastly, for the third replication all initial and final foot T were obtained.

Behaviour Analyses: Videos were transcribed by a single observer using a scan sampling technique at five-minute intervals for various activity, thermoregulatory, pecking, and other behaviours (described in Table 2.2). At each time point all eight birds within the crate were observed, and their behaviour was recorded. If a bird was not visible due to being blocked by other birds within the crate and their behaviour could not be characterized, it was categorized as unidentifiable. Individual birds were not identified. Behaviour is represented as percent frequency.

Table 2. 2. Ethogram of behavioural activities monitored during the study and the criteria for each behaviour. All behaviours are mutually exclusive, except for panting (Webster and Hurnik, 1990; Hurnik et al., 1995; Webster 2000; Henrikson et al., 2018).

Category	Behaviour	Description
Activity	Motionless	Bird may be standing or is in a crouched position where its body is in contact with the floor of the crate (difficult to determine via videos). Bird is motionless and may be in a collected posture with its head retracted, and eyes may be open or closed
Activity	Active	Bird is moving feet and/or wings, and is changing position/location within the crate
Activity	Shuffle	Bird is moving feet while moving its body side to side. Minimum of one series of side to side movements to be considered. Bird resets itself near the same position as when behaviour began.
Thermoregulatory	Burrow	Birds are actively attempting to burrow underneath other birds
Thermoregulaotry	Shiver	Repeated quivering of the wings and/or body in order to produce body heat
Thermoregulatory	Ptiloerection	Feathers are erect or being ruffled in an organized manner
Thermoregulaotry	Panting	Increase in the breathing of the bird characterized by an open mouth, polypnea, or increased thoracic movements
Thermoregulaotry	Survey	Movements of the bird's head suggesting surveillance of the bird's environment
Pecking	Bird Pecking	Bird is using the beak to, in short and quick forward motions of the head, make contact with other birds
Pecking	Object Pecking	Bird is using the beak to, in short and quick forward motions of the head, make contact with either the crate or with the sensors on the crate
Other	Preen	Beak is used to manipulate the feathers on the bird's own body
Other	Head shake	Rapid side-to-side movements of the head. Bird is immobile. Minimum of two series of side to side movements to be considered
Other	Gulp	Head of bird is pointed vertically upward, beak is opened, and bird takes a large gasp of air
Other	Scratching	Bird raised its leg over its wing and repeatedly rubs its head with its talons
Other	Unidentifiable	Bird cannot be seen, or behavior cannot be characterized

2.3.8 Statistical Analyses

The experiment was designed as a 5x2 factorial arrangement, in a randomized complete block design (farm of origin as block), of T/RH combination, and D. There were three replications per T/RH combination, and D. The experimental unit in this research experiment was crate. Data were checked for normality prior to analyses using Proc Univariate and were log transformed when necessary. Data were analyzed in a one-way ANOVA using the Proc Mixed procedure (SAS® 9.4, Cary, NC) with means separation conducted using the Tukey test. Differences were considered significant at $P \leq 0.05$ and trends were noted when $0.05 < P \leq 0.10$.

2.4 Results

Core body temperature: There were no interactive effects between the T and RH combinations, and D on pullet CBT. The change in the CBT of pullets exposed to each T/RH group over the four hour duration is presented in Figure 2.6, and the eight hour duration in Figure 2.7, though the results presented in these figures were not statistically analyzed. To highlight, in Figure 2.6, pullets exposed to the -15 treatment for the four hour D had a decrease in their Δ CBT which lasted over the entire D. However, pullets exposed to the -15 treatment for the eight hour D had an initial decrease in their Δ CBT, but by the end of the exposure D, pullet were able to stabilize their CBT (Figure 2.7). To note in the -15 treatment, the mean CBT of pullets in the four- and eight-hour D decreased by a maximum of 2°C and 2.6°C, respectively, by 1.75 hours of exposure. Additionally, pullets exposed to the 30/80 treatment for both the four and eight hour D had an initial spike in their Δ CBT, however, this plateaued over time (Figure 2.6-2.7). After 45 minutes of exposure to the 30/80 treatment, pullets within the four- and eight-hour D had a maximum increase in their CBT of approximately 1.3°C and 1.5°C, respectively. Lastly, differing from the four hour D, pullets exposed to the 21/30 treatment had an increase (approximately 1.3°C) in their Δ CBT by the end of the eight hour D (Figure 2.6). With regards to the last hour of exposure, pullets in the -15 treatment had a significantly lower CBT compared to all other treatments (Table 2.3); however, when Δ CBT was calculated there was only a tendency towards significance. There was an effect of D on pullet Δ CBT, with a minor decrease as a result of the four hour D, and a minor increase as a result of the eight hour D (Table 2.3). No effect of duration was observed for pullet CBT or baselines temperatures.

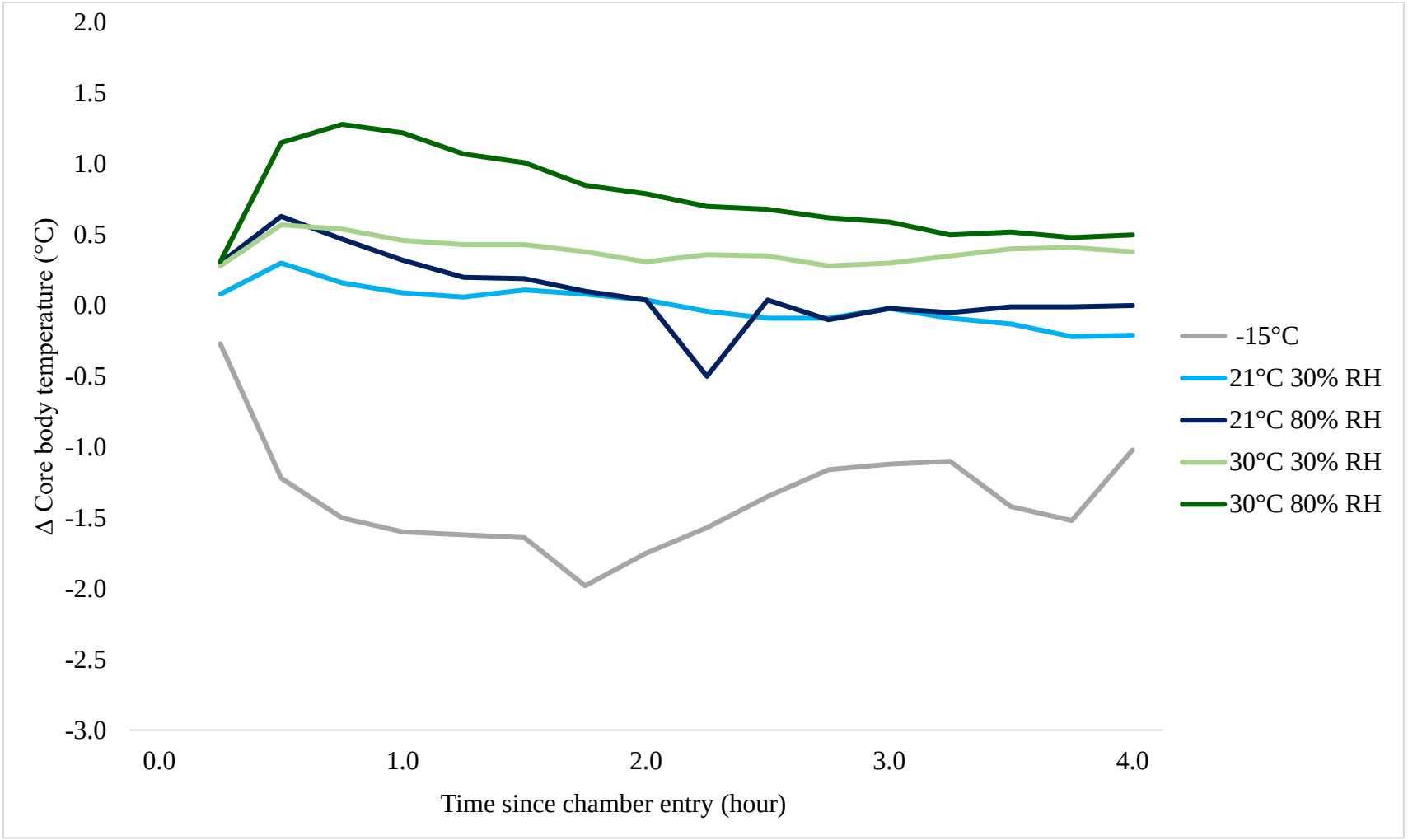

Figure 2. 6. Change in the core body temperature (CBT) of white-feathered layer pullets when exposed to various temperature and relative humidity combinations for a four-hour duration (n=three replications, eight pullets per replication).
ΔCBT (°C) = mean pullet CBT – mean baseline CBT

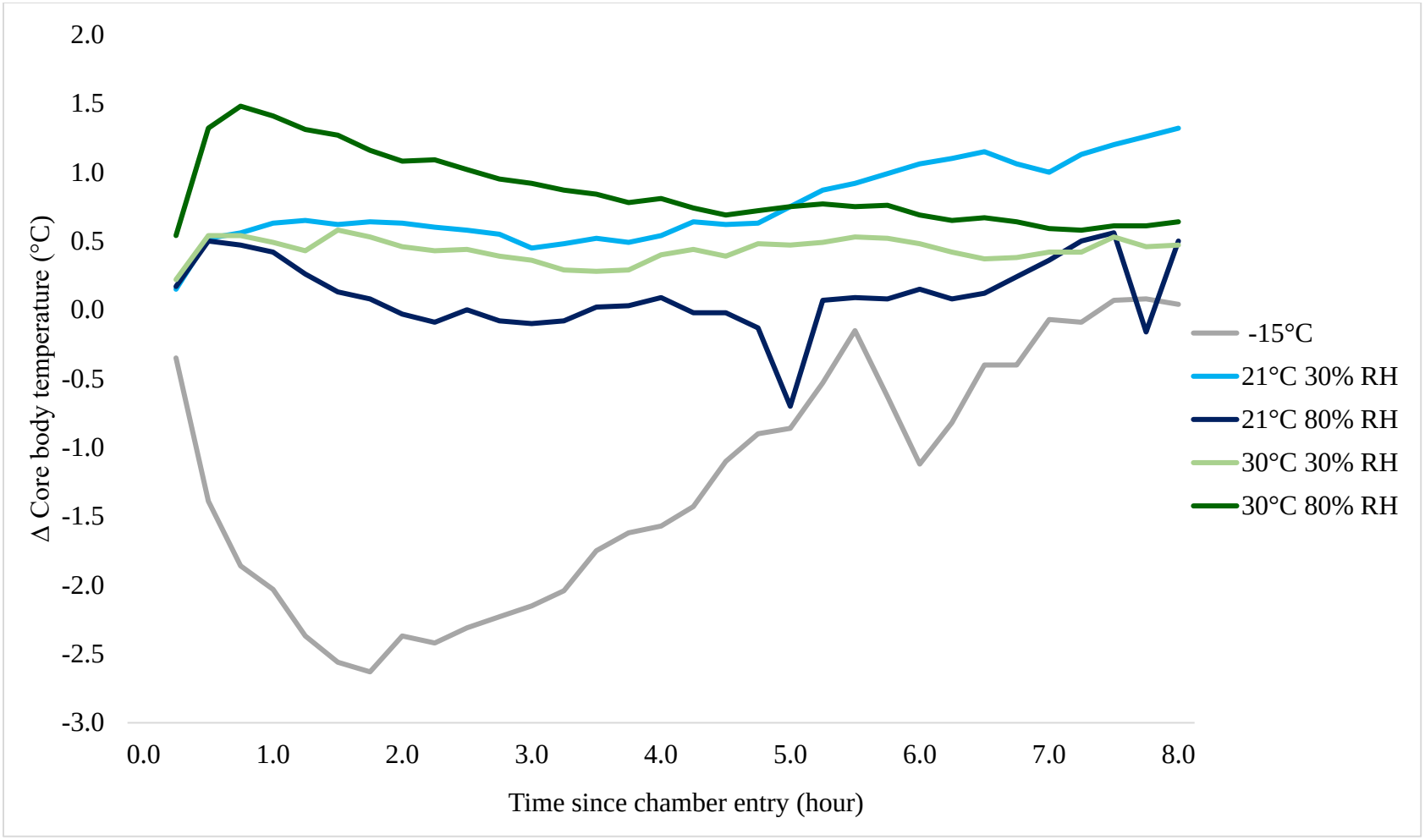

Figure 2. 7. Change in the core body temperature (°C) of white-feathered layer pullets when exposed to various temperature and relative humidity combinations for an eight-hour duration (n=three replications, eight pullets per replication)
ΔCBT (°C) = mean pullet CBT – mean baseline CBT

Table 2. 3. Effects of various temperature (T) and relative humidity (RH) combinations (n=6), at two different time durations (D) (n=15), on the change in core body temperature (CBT) of white-feathered layer pullets from the last hour of exposure.

	T/RH Combinations						D				
Parameter (°C)	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[1]
Pullet CBT	**38.24^{b}**	**40.00^{a}**	**39.91^{a}**	**40.61^{a}**	**40.75^{a}**	**<0.001**	39.64	40.15	0.124	0.489	0.236
Baseline	**38.86^{b}**	**39.47^{ab}**	**39.70^{ab}**	**40.18^{a}**	**40.19^{a}**	**0.034**	39.75	39.62	0.624	0.846	0.169
Δ CBT	-0.62	0.53	0.21	0.43	0.56	0.079	**-0.11^{b}**	**0.53^{a}**	**0.025**	0.430	0.163

[1] Pooled standard error of mean

Delta (Δ) CBT = mean pullet CBT - mean baseline CBT

-15=-15°C, uncontrolled RH, 21/30=21°C,30%RH, 21/80=21°C,80%RH, 30/30=30°C,30%RH, 30/80=30°C,80%RH

Blood Physiology: There were no interactions between the T/RH and D on pullet blood physiology examined in this study. No effect of the T/RH treatment combinations were noted on pullet blood pH, BEecf, pCO_2, tCO_2, bicarbonate, pO_2, sO_2, blood sodium, or the hematocrit and hemoglobin levels (Table 2.4 - 2.7). Blood glucose was influenced by the T/RH conditions (Table 2.4), where final concentrations were higher in pullets exposed to the 30/80 treatment compared to those exposed to either of the treatments at 21°C.

There was no impact of duration on pullet blood glucose, pH, BEecf, pO_2, sO_2, blood sodium or, the hematocrit and hemoglobin levels (Table 2.4, Table 2.6, and Table 2.7). However, increased exposure D altered pullet blood pCO_2, tCO_2, and bicarbonate, with levels being lower in pullets exposed to the eight-hour D, compared to the four-hour D (Table 2.5).

Heterophil to lymphocyte (H/L) ratio: There were no interaction effects between the T/RH combinations and D on the pullet's H/L ratio. The T/RH combinations had no impact on the pullet's H/L ratio (Table 2.8). Finally, pullet H/L ratio was not impacted by the exposure D (Table 2.8).

Foot temperatures: In this study there was no interaction impacts of T/RH and D on pullet foot temperatures. Final foot T were lowest in pullets exposed to the -15 treatment and were higher in pullets exposed to the 30/80 treatment compared to those exposed to the cold and neutral conditions (Table 2.9). There was no impact of exposure D on the foot T of pullets in this study.

Behaviour: There were no interactions between T/RH and D on pullet behaviour during exposure. Pullet behaviour is represented as a percent of time birds spent performing the respective behaviour and is presented in Table 2.10. No effect of either exposure conditions, or D was noted on the percent of time performing object pecking, burrowing, shivering, ptiloerection, head shaking, scratching, shuffling, or unidentifiable behaviours. Pullets in the -15 treatment, along with the 21/30 treatment, spent the more time motionless, compared to those exposed to the 30/30 and 30/80 treatments. Compared to pullets in the -15 treatment, pullets in 30/80 treatment spent more time active. Panting was observed to occur more frequently in the pullets in the 30/30 and 30/80 treatments, compared to those in the other treatments. In both the 30/30 and 30/80 treatments, pullets spent more time surveying compared to those in the -15 treatment. Bird pecking occurred more often in pullets in the 30/30 treatment compared to that of the -15 and

Table 2. 4. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the blood pH, base excess in the extracellular fluid (BEecf), and blood glucose levels of white-feathered layer pullets.

	T/RH Combinations						D				
Parameter	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[1]
Initial Glucose (mmol/L)	13.16	13.19	13.18	13.23	13.34	0.979	13.29	13.16	0.526	0.793	0.101
Final Glucose (mmol/L)	**13.03^{ab}**	**12.57^{b}**	**12.53^{b}**	**12.98^{ab}**	**13.79^{a}**	**0.007**	13.05	12.91	0.466	0.401	0.146
Δ Glucose (mmol/L)	-0.13	-0.62	-0.65	-0.25	0.45	0.134	-0.24	-0.25	0.952	0.312	0.155
Initial pH	7.11	7.12	7.11	7.10	7.09	0.863	7.11	7.11	0.653	0.820	0.008
Final pH	7.16	7.15	7.17	7.18	7.16	0.258	7.16	7.16	0.645	0.971	0.004
Δ pH	0.05	0.03	0.06	0.08	0.07	0.471	0.05	0.05	0.494	0.840	0.008
Initial BEecf (mmol/L)	-9.39	-8.50	-9.00	-10.33	-10.44	0.437	-10.00	-9.07	0.234	0.769	0.369
Final BEecf (mmol/L)	-8.56	-6.89	-6.44	-6.86	-7.33	0.260	-6.94	-7.49	0.379	0.920	0.413
Δ BEecf (mmol/L)	0.83	1.61	2.56	3.47	3.11	0.181	3.06	1.58	0.059	0.815	0.472

[1] Pooled standard error of mean

Delta (Δ) = final – initial

-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 5. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the blood gases and bicarbonate of white-feathered layer pullets.

Parameter	T/RH Combinations					*P*-Value	D		*P*-Value	Interaction *P*-Value	SEM[1]
	-15	21/30	21/80	30/30	30/80		4 hours	8 hours			
Initial pCO_2 (mmHg)	62.97	65.35	64.52	62.63	63.63	0.945	61.55	66.09	0.069	0.641	1.219
Final pCO_2 (mmHg)	56.49	62.73	60.63	57.51	59.56	0.250	60.56	58.21	0.216	0.972	1.019
Δ pCO_2 (mmHg)	-6.48	-2.62	-3.89	-5.12	-4.07	0.942	**-0.99[a]**	**-7.88[b]**	**0.034**	0.680	1.770
Initial tCO_2 (mmol/L)	21.94	22.94	22.56	21.25	21.33	0.404	21.39	22.62	0.069	0.683	0.333
Final tCO_2 (mmol/L)	21.22	23.78	23.89	23.14	23.06	0.168	23.46	22.78	0.241	0.917	0.400
Δ tCO_2 (mmol/L)	0.22	0.84	1.33	1.89	1.73	0.406	**2.07[a]**	**0.16[b]**	**0.019**	0.771	0.502
Initial HCO_3^- (mmol/L)	20.08	21.00	20.51	19.35	19.39	0.381	19.53	20.60	0.096	0.706	0.310
Final HCO_3^- (mmol/L)	20.03	21.89	22.07	21.50	21.30	0.211	21.67	21.05	0.281	0.911	0.384
Δ HCO_3^- (mmol/L)	0.05	0.89	1.56	2.15	1.91	0.256	**2.14[a]**	**0.45[b]**	**0.019**	0.754	0.462

[1] Pooled standard error of mean
pCO_2 = partial pressure of carbon dioxide
tCO_2 = total carbon dioxide
HCO_3^- = bicarbonate
mmHg = millimeters of mercury
mmol/L = Millimoles per liter
Delta (Δ) = final – initial
-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 6. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the blood pO_2 and sO_2 of white-feathered layer pullets.

	T/RH Combinations						D				
Parameter	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[1]
Initial pO_2 (mmHg)	67.94	67.28	66.72	69.31	66.94	0.813	67.06	68.22	0.424	0.604	0.851
Final pO_2 (mmHg)	80.56	71.94	69.06	69.14	69.50	0.445	69.83	74.24	0.355	0.626	2.342
Δ pO_2 (mmHg)	12.62	4.66	2.34	-0.17	2.56	0.409	2.77	6.02	0.565	0.631	2.215
Initial sO_2 (%)	84.97	84.56	84.28	84.53	83.11	0.711	84.19	84.39	0.828	0.675	0.432
Final sO_2 (%)	89.44	87.94	87.39	87.75	87.39	0.584	87.66	88.31	0.483	0.888	0.485
Δ sO_2 (%)	4.47	3.38	3.11	3.22	4.28	0.951	3.47	3.92	0.758	0.685	0.557

[1] Pooled standard error of mean
pO_2 = partial pressure of oxygen
sO_2 = soluble oxygen
mmHg = millimeters of mercury
Delta (Δ) = final – initial
-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 7. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the blood sodium (Na) levels, hematocrit, and hemoglobins levels of white-feathered layer pullets.

Parameter	T/RH Combinations					*P*-Value	D		*P*-Value	Interaction *P*-Value	SEM[1]
	-15	21/30	21/80	30/30	30/80		4 hours	8 hours			
Initial Na (mmol/L)	144.36	144.00	143.83	144.36	144.17	0.932	144.05	144.23	0.712	0.774	0.351
Final Na (mmol/L)	140.31	143.17	143.56	145.00	144.28	0.465	143.64	142.88	0.604	0.398	0.842
Δ Na (mmol/L)	-4.05	-0.83	-0.27	0.64	0.11	0.401	-0.41	-1.35	0.384	0.377	0.799
Initial Hematocrit (%PCV)	25.25	24.67	25.83	26.42	25.78	0.364	25.63	25.64	0.961	0.636	0.289
Final Hematocrit (%PCV)	23.33	22.67	23.83	24.75	23.83	0.426	23.59	23.89	0.782	0.460	0.360
Δ Hematocrit (%PCV)	-1.92	-2.00	-2.00	-1.67	-1.95	0.980	-2.04	-1.75	0.709	0.860	0.240
Initial Hemoglobin (mmol/L)	5.35	5.26	5.46	5.57	5.45	0.392	5.42	5.42	0.949	0.649	0.059
Final Hemoglobin (mmol/L)	4.93	4.79	5.04	5.23	5.04	0.426	4.98	5.06	0.718	0.484	0.077
Δ Hemoglobin (mmol/L)	-0.42	-0.47	-0.42	-0.34	-0.41	0.963	-0.44	-0.36	0.581	0.854	0.052

[1] Pooled standard error of mean
PCV = Pack cell volume
Delta (Δ) = final – initial
-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 8. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the heterophil to lymphocyte (H/L) ratio of white-feathered layer pullets.

	T/RH Combinations						D				
Parameter	-15	21/30	21/80	30/30	30/80	P-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[1]
Initial H/L Ratio	0.41	0.38	0.39	0.46	0.47	0.103	0.44	0.40	0.269	0.657	0.027
Final H/L Ratio	0.60	0.51	0.53	0.50	0.66	0.319	0.60	0.53	0.175	0.855	0.028
Δ H/L Ratio	0.19	0.13	0.14	0.04	0.19	0.581	0.16	0.13	0.856	0.646	0.025

[1] Pooled standard error of mean

Delta (Δ) = final – initial

-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 9. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15), on the foot temperatures of white-feathered layer pullets.

Parameter (°C)	T/RH Combinations						D				
	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[1]
Initial Foot Temperature	28.25	32.42	29.11	30.45	28.73	0.197	30.00	29.18	0.323	0.209	0.660
Final Foot Temperature	**12.97^{d}**	**30.18^{c}**	**32.48^{b}**	**34.45^{ab}**	**35.20^{a}**	**<0.001**	27.47	27.75	0.303	0.751	2.019
Δ Foot Temperature	-15.28	-2.24	3.37	4.00	6.47	0.113	-2.53	-1.43	0.648	0.971	2.018

[1] Pooled standard error of mean

Delta (Δ) = final – initial

-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Table 2. 10. Percent of time white-feathered layer pullets spent performing various behaviours during exposure to various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (D) (n=15).

Category	Behaviour (% of time)	T/RH Combinations					*P*-Value	D		*P*-Value	Interaction *P*-Value	SEM[1]
		-15	21/30	21/80	30/30	30/80		4 hours	8 hours			
Activity	Motionless	**93.46^{a}**	**93.35^{a}**	**90.10^{ab}**	**79.80^{b}**	**68.45^{c}**	**<0.001**	85.43	84.63	0.745	0.845	2.033
Activity	Active	**0.12^{b}**	**0.49^{ab}**	**0.99^{ab}**	**0.63^{ab}**	**1.82^{a}**	**0.009**	**1.07^{a}**	**0.55^{b}**	**0.033**	0.864	0.179
Activity	Shuffle	0.34	0.80	1.41	0.95	0.94	0.464	0.82	0.95	0.849	0.584	0.156
Thermo-regulatory	Burrow	0.07	0	0	0.02	0	0.528	0	0.04	0.204	0.528	0.015
Thermo-regulatory	Shiver	0.10	0	0.10	0.02	0	0.599	0	0.09	0.155	0.599	0.027
Thermo-regulatory	Ptiloerection	0.07	0	0	0.02	0.09	0.666	0.04	0.04	0.704	0.337	0.023
Thermo-regulatory	Panting	**0^{b}**	**0.23^{b}**	**0.30^{b}**	**9.41^{a}**	**18.22^{a}**	**<0.001**	5.07	6.20	0.513	0.958	1.731
Thermo-regulatory	Survey	**1.24^{b}**	**2.15^{ab}**	**2.27^{ab}**	**3.18^{a}**	**4.11^{a}**	**0.001**	2.76	2.42	0.342	0.876	0.248
Pecking	Object Peck	0.05	0.37	0.02	0.38	0.32	0.066	0.23	0.23	0.952	0.883	0.056
Pecking	Bird Peck	**0^{b}**	**0.16^{ab}**	**0^{b}**	**0.29^{a}**	**0.16^{ab}**	**0.045**	0.10	0.15	0.472	0.774	0.035
Other	Preen	**0.29^{b}**	**0.78^{ab}**	**1.46^{a}**	**1.19^{a}**	**0.82^{ab}**	**0.018**	1.01	0.81	0.247	0.885	0.116
Other	Head Shaking	0.19	0.28	0.54	0.38	0.07	0.171	0.32	0.26	0.536	0.986	0.064
Other	Gulp	**0.10^{ab}**	**0.44^{a}**	**0.43^{a}**	**0.23^{ab}**	**0.05^{b}**	**0.032**	0.20	0.29	0.328	0.129	0.057
Other	Scratch	0	0.14	0.07	0.22	0.19	0.327	0.10	0.15	0.530	0.587	0.039
Other	Unidentifiable	4.01	1.00	2.31	3.31	4.95	0.227	2.93	3.30	0.478	0.661	0.748

[1] Pooled standard error of mean

-15 = -15°C, uncontrolled RH, 21/30 = 21°C,30%RH, 21/80 = 21°C,80%RH, 30/30 = 30°C,30%RH, 30/80 = 30°C,80%RH

Behaviour was recorded using five minute scan sampling for the entire treatment period (four or eight hours) and presented in percent frequency

30/80 treatments, pullets spent more time surveying compared to those in the -15 treatment. Bird pecking occurred more often in pullets in the 30/30 treatment compared to that of the -15 and 21/80 treatments. Pullets in the -15 treatment spent less time preening compared to pullets in the 21/80 and 30/30 treatments. In both treatments at 21°C, pullets were found to perform gulping behaviours more often compared to pullets in the 30/80 treatment. There was no effect of exposure D on pullet behaviours including motionless, shuffling, burrowing, shivering, ptiloerection, panting, surveying, object pecking, bird pecking, preening, head shaking, gulping, scratching, or unidentifiable behaviours. An effect of D was only observed on levels of activity, in which pullets in the eight hour D spent less time active compared to the pullets in the four hour D.

2.5 Discussion

The microclimate (combination of T and RH) within the transport trailer can influence the CBT of birds during transport, which may negatively impact their welfare. Pullet CBT, collected in this experiment over time, demonstrated an initial depression or spike early in the exposure to either the -15 or 30/80 treatment, respectively. However, interestingly, the data also demonstrates that under the 30/80 conditions, by the end of both the four- and eight-hour D, the CBT plateaued at a level closer to the baseline. Additionally, the data collected indicate that the CBT of pullets exposed to the -15 treatment rebounded by the end of the eight-hour D. This suggests that through the implementation of behavioural and physiological mechanisms, pullets were either able to stabilize their CBT or avoid a further decrease in their CBT and cope with the environmental conditions. Finally, in both the four and eight hour D, pullets exposed to the 21/80 treatment experienced abrupt drops in CBT, however, these drops quickly rebounded. These abrupt depressions in CBT, though interesting to note, are not well understood.

The data from the current experiment indicate that during the last hour of exposure to the -15 treatment, pullets had a significantly lower CBT. The results from a study by Dadgar et al. (2012a) agree with that of the current study. Dadgar et al. (2012a) noted that broilers (five to six weeks of age) exposed to -14°C for a three-hour D had a lower CBT compared to those exposed to either -9°C or 21°C for the same D. Dadgar et al. (2010) also noted a lower CBT in broilers (39-42 days of age) exposed to -7°C for a three to four hour D, compared to those exposed to T above 5°C. However, not all previous research agrees with that of the current study. Henrikson et al. (2018) examined the change in CBT of turkey toms (16 weeks of age) during the last hour of

exposure to cold conditions (-18°C for an eight-hour D). They found no differences compared to toms exposed to neutral conditions (20°C with 30%RH, or 20°C with 80%RH) for the same D. Differences between previous research and the current study may be attributed to species differences, as well as differences in feathering between 16-week old turkey toms and layer pullets.

Though there was no impact of the T/RH conditions on pullet Δ CBT, there was an effect of D. The current study showed that a change in pullet CBT resulted from an increased transport D. Lin et al. (2005) observed that broilers (one week of age) transported at 30°C with 60%RH had a lower rectal T after 16 hours of exposure compared to broilers exposed to the same conditions for a D of one hour. However, at one week of age, broilers have a limited ability to thermoregulate, which may have influenced the results of the mentioned studies (Freeman, 1976; Lin et al., 2005). Conversely, Menten et al. (2006) noted that broilers exposed to 35°C with 85%RH for 90 and 120 minutes had a significantly higher rectal T compared to broilers that were not exposed (control group) and compared to broilers exposed to the same conditions for a 30 or 60 minutes. Both of the previous studies used rectal thermometers to measure the bird's temperature, whereas in the current study miniature loggers were ingested, which recorded either crop or gizzard T as a measure for CBT. Therefore, T between the current study and that of previous research may differ. In the current study, by the last hour of both the four- and eight-hour duration, there was a minimal decrease or increase in the Δ CBT, respectively. This can indicate that pullets, through the implementation of behavioural and physiological mechanisms, were able to cope with the environmental conditions. An increased exposure D may be a concern for bird welfare during transportation, but in the current study, a D of up to eight hours had minimal impact on the pullet's Δ CBT. Previous research has indicated that the normal CBT of a bird can fluctuate by ±1°C, and differences in the current study due to increased exposure D did not deviate by more than 0.6°C (Donkoh, 1989; Sandercock et al., 2001; Knezacek, 2005; Dadgar et al., 2010).

Thermoregulation against heat or cold stress can be energy-demanding (Sherwood et al., 2013). Pullets exposed to the 30/80 treatment had to implement behavioural and physiological mechanisms in order to maintain homeostasis in this condition. Therefore, along with maintaining normal metabolic functions, pullets were required to mobilize more glucose stores to support these thermoregulatory mechanisms, resulting in a higher final concentration of blood

glucose. On the other hand, pullets in both of the neutral conditions in the current study may not have been required to thermoregulate against the environmental conditions. Therefore, it may be suggested that due to pullets not having access to feed during exposure, and as a result of normal metabolic activity, blood glucose concentrations would decrease in pullets exposed to these treatments. In this study, blood glucose concentrations of the pullets may have been impacted, as pullets were not feed restricted before exposure to the treatment conditions, resulting in potentially more available glucose in the blood upon exposure to thermal stress. Vosmerova et al. (2010) exposed broilers (42 days old, and unknown feed restriction) to hot (25-35°C), neutral (10-20°C), and cold conditions (-5-5°C) and transported them for a distance of 130 kilometers inside a commercial truck. The authors found that broilers exposed to 25-35°C during transportation had higher concentrations of blood glucose (13.35 mmol/L), compared to those exposed to 10-20°C (12.41 mmol/L). These results support the conclusions made from the current study, in that birds mobilize energy stores in order to thermoregulate against heat stress.

Mechanisms used by birds to dissipate heat, such as evaporative heat loss, can cause changes in the levels of blood gases, as well as the acid-base balance in the body (Ait-Boulahsen, 1989; Sherwood et al., 2013; Khosravinia, 2017). Though panting was increased in pullets exposed to both 30°C conditions, exposure to these conditions did not affect levels of blood gases, or acid-base balance parameters in the body. However, there was an effect of exposure D on the blood pCO_2, tCO_2, and bicarbonate. Although not significantly different, pullets exposed for the eight hour D numerically panted for a longer period of time, causing a decreased levels of pCO_2, tCO_2, and bicarbonate in the blood. Decreased levels of these parameters can indicate a shift towards a respiratory alkalosis which can negatively impact the welfare of birds; however, these decreases in blood gases and acid-base parameter were not enough to influence the blood pH of the pullets. Additionally, increased exposure D and increased time spent hyperventilating was not enough to impact the blood pO_2, sO_2, or BEecf. There is very little information regarding the effect of various transportation D on the blood physiology of poultry. A study by Ait-Boulahsen et al. (1989) fasted and exposed five to six week old broilers for various D (0 hours fasting with 124 minutes of exposure, 24 hours fasting with 194 minutes exposure, and 72 hours fasting with 230 minutes exposure), to a stepwise chamber T increase (29 to 37°C over four hours, then 37 to 41°C maintained over four hours). No effect was noted on blood pCO_2; however, blood concentrations of bicarbonate were lower with increasing fasting and exposure

D. Various factors, such as exposure conditions, fasting duration, and exposure duration, could have influenced the results in this study (Ait-Boulahsen et al., 1989). A study by Sandercock et al. (2001) exposed 35- and 63-day old broilers to either heat stress (32°C, 75%RH) or neutral conditions (21°C, 50%RH) for a two-hour D, and found that at both ages, the pCO_2 was lower in the birds exposed to both the heat stress and the neutral conditions. The results of previous research vary compared to that of the current study where an increased transportation D had an impact on the blood gases of the pullets, but this impact was not enough to change the pH of their blood. Therefore, a D of up to eight hours had minimal impacts on the blood physiology of pullets in this study.

During transportation, birds would not have access to water, which can result in them becoming dehydrated. One way to evaluate dehydration during transportation is by examining the levels of sodium and hemoglobin in the blood, and evaluating the hematocrit (Khosravinia, 2017, Minka and Ayo, 2017). As a result of defecation, it may be expected that blood sodium levels would decrease, while as a result of dehydration, the hemoglobin and hematocrit levels would increase (Thomas et al., 2008; Bergoug et al., 2013). However, pullets in the current study were provided water up until the pre-treatment procedures, therefore, neither the T/RH conditions nor an increased exposure D was enough to cause changes in the dehydration markers. To the author's knowledge, there has been minimal research conducted examining the effects of thermal stress during transportation on dehydration markers in birds. A study by Khosravinia (2017) observed that blood sodium levels in broiler chicks were lower in those transported (27-29°C with 35-46%RH) for 800 kilometers compared to those transported for less than 200 kilometers. However, the broiler chicks would have still had yolk reserves, which could have influenced the results of this study (Khosravinia, 2017). Borges et al. (2004) subjected broilers to a stepwise increase in T (24-41°C) for 11 hours and found that there were no differences in the hematocrit or hemoglobin in the birds.

An increase in the H/L has been recognized as an indicator of chronic stress in poultry (Lin et al., 2006). Previous research exposing birds to thermal stress has found results that contradict that of the current study. Aksit et al. (2006) exposed broilers to either 15°C, 22°C, or 34°C, for two hours and noted a higher H/L ratio in the broilers exposed to 34°C (0.81) compared to the broilers exposed to 15°C (0.40) or 22°C (0.30). However, the broilers used in the mentioned study were also exposed to various environmental conditions (22°C, 22-28°C, or

34°C) in a prior consecutive study, which may have influenced the results (Aksit et al., 2006). Furthermore, a study conducted by Altan et al. (2010) measured the change in the H/L ratio in broilers after exposure to heat stress. In their study, broilers were exposed to 38°C for three hours in their home pen at 35 and 36 days of age (feed was removed for the exposure period) (Altan et al., 2010). These broilers were noted to have a higher H/L ratio compared to broilers not exposed to the heat stress; however, birds were able to remain in their home pens, therefore they would not have experienced the same stressors as during transportation (Altan et al., 2010). The results of previous research may differ from that of the current study due to differences in strains, age, exposure conditions, and D of exposure. In the present study, there were no impacts of either the T/RH conditions, or exposure D on pullet H/L ratio. It may be hypothesized that the T/RH conditions may not have been enough to cause a chronic stress response, or the exposure D was not long enough to observe a change in the pullets H/L ratio; however, the reason for there being no effect is not well known.

Radiation or convection by means of the bare skin of the extremities can be a mechanism by which birds dissipate heat when exposed to hot temperatures. In the present study, pullet foot T were recorded to examine the impact of exposure to hot T on the extremities, and the data indicate that heat stress affected foot temperatures. Higher foot T in the pullets subjected to heat exposure suggests vasodilation to the extremities, which can aid in dissipating heat from the core (Whittow et al., 1964; Richards, 1971). As a result of heat exposure, vasodilation can occur as a mechanism by which to increase blood flow to the extremities and dissipate heat through the bare skin (Richards, 1971; Whittow, 1964). Richards (1971) exposed hens to a preliminary treatment (13-15°C for one hour), and then exposed the hens to either 20°C, 30°C, or 40°C for three hours. This study found that hens exposed to 30°C had a toe T of 35°C, and a comb T of 37°C post-exposure (Richards, 1971). Furthermore, Rector et al. (1952) noted that birds exposed to 32-35°C had a comb T of approximately 35-38°C; however, the T within the exposure room was not controlled, and room T and comb T were recorded simultaneously, leading to inconsistent T exposure. The results from these studies coincide with that of the current study, where final foot T of pullets were highest after exposure to the high T, high RH treatment. Higher pullet foot T in the current study as a result of heat exposure suggest vasodilation to the extremities as an attempt to dissipate heat through the bare skin.

On the other hand, lower foot T in the pullets subjected to cold exposure can suggest vasoconstriction of the blood vessels to the extremities (Wellehan, 2014). As an adaptive response to cold T, blood vessels will vasoconstrict to limit blood flow to the extremities in order to conserve heat for the organs in the core (Wellehan, 2014). There has been minimal research observing the changes in the extremity T of poultry when exposed to cold stress. Millard (1974) observed that blood flow to the dorsal pedal artery of the giant petrel decreased after 40 seconds of being submerged in ice water, after which pronounced vasoconstriction was noted. However, the giant petrel is an arctic species, thus they may have a greater adaptation to a cold environment compared to commercial layer pullets. Therefore, comparison to the current study may be difficult. Frostbite of human skin has been noted to occur at a skin temperature of approximately -2.2°C due to the salinity of the body fluids; however, the final foot T for the pullets in the current study were approximately 13°C, which may still be above a concerning T for the susceptibility of frostbite (Nagpal and Sharma, 2004).

When exposed to cold T, the bird's first response will be to change their behavioural patterns in order to conserve heat for the core. Birds will typically huddle and burrow under conspecifics to minimize the amount of body surface area exposed to the environment, which can conserve heat and energy (Mount, 1979; Strawford et al., 2011). Pullets in the current study were able to conserve heat and energy by spending more time motionless. However, the performance of thermoregulatory behaviours associated with cold exposure such as burrowing and shivering were not different in the present study, suggesting that pullets were able to cope with the cold stress through minimal behavioural changes. A study by Henrikson et al. (2017) found that turkey hens and toms spent more time huddling and performing ptiloerection when exposed to cold T (-18°C) for three hours. The results of the mentioned study indicate that birds exposed to cold conditions will spend more time performing thermoregulatory behaviours that aim to conserve body heat, which is in agreement with the results found in the current study. However, unlike the results of the current study, Strawford et al. (2011) exposed male (32 days of age) and female (33 days of age) broilers to -5, -10, and -15°C for a three-hour D while inside transportation crates, and found that overall space usage inside the crates did not differ compared to when birds were crated pre-exposure. Additionally, burrowing activity did not differ, but the broilers were observed moving away from locations in the crates where drafts were present during exposure (Strawford et al., 2011). There has been limited research observing the

behaviour of birds exposed to cold T, and differences in results can be due to species differences, as well as differences in environmental conditions, and exposure D.

Preening may be described as a comfort behaviour, but during situations of stress or frustration, it can also be categorized as a displacement behaviour (Webster, 2000; Barnett, 2007; NFACC, 2017). Exposure to the varying T/RH conditions during the simulated transportation likely introduced stressors for the pullets, which may suggest that preening in this situation was performed as a displacement behaviour. The results presented by Henrikson et al. (2018) contradict those of the current study, who found that turkey hens exposed to -18ºC (eight hour D) preened more often compared to those exposed to either 20ºC with 30%RH, or 20ºC with 80%RH. Furthermore, Cheng and Jefferson (2008) noted that after two hours of transportation (environmental conditions not mentioned), laying hens (17 weeks of age) were noted to preen significantly less compared to before transportation. In the current study, the underlying reason behind the pullets preening more in the 21/80 and 30/30 treatments is not known, as preening can be performed under various conditions; however, in the present study, preening was performed in a low frequency.

There is minimal literature examining the performance of bird pecking or gulping behaviours with regards to exposure to heat or cold stress. Bird pecking has been suggested to be an aggressive, stereotypic, or exploratory behaviour, but this primarily pertains to studies conducted in pens or cages rather than during transportation (Webster and Hurnik, 1990; Webster, 2000). To the author's knowledge, there has been limited research on gulping behaviours in poultry; therefore, the purpose behind performing this behaviour is not well known. However, both bird pecking and gulping behaviours in the current study were observed in a low frequency. Therefore, they do not appear to have any biological relevance to either of these behaviours.

As a result of heat exposure in the current study, pullets spent more time performing thermoregulatory behaviours aimed to dissipate heat. The main mechanism for poultry to dissipate heat is through evaporative heat loss (Mount, 1979); therefore, it would be expected that pullets exposed to both of the high-T treatments (30°C) in the current study would have an increased respiratory frequency. This is supported by the results presented by Menten et al. (2006) who noted an increased respiratory frequency in broilers (42 days of age) exposed to heat stress (35°C with 85%RH) for 60 minutes (51 breaths per minute) compared to broilers not

exposed to the heat stress (31 breaths per minute). Toyomizu et al. (2005) also noted that when 21 day-old broilers were subjected to 38°C (120 minute D), by 60 minutes of exposure the respiratory frequency increased (261 breaths per minute) compared to before heat exposure (61 breaths per minute), however, by 90 minutes of heat exposure the respiratory frequency significantly declined. Though a significant difference was noted during heat exposure in the mentioned study, the methodology for recording and analyzing the respiratory frequency of the broilers was not described. Finally, Tamzil et al. (2013) found that the average respiratory frequency of laying hens exposed to 40°C was higher after one and a half hours of exposure, compared to half an hour and one hour of exposure; however, in this study, the respiratory frequency was only recorded during the last five minutes of the respective exposure D and was recorded using a handheld counter (Tamzil et al., 2013). Though there are species and methodology differences between the aforementioned studies and the current study, they all agree that when birds are exposed to heat stress, they will pant more in order to dissipate heat.

Surveying can be defined as increased vigilance in birds, as well as birds moving their heads within, or potentially through the crate. Surveying can increase the amount of body surface area exposed to the environment and allow greater dissipation of heat through non-evaporative heat loss. The bare skin can dissipate heat through radiation and convection, which can help birds in maintaining their CBT (Mount, 1979). Henrikson et al. (2017) observed turkey toms and hens exposed to cold T (-18°C) and neutral conditions (20°C with 30%RH and 20°C with 80%RH) and found no difference in surveying behaviours in either toms or hens. In the current work, birds exposed to both 30-degree treatments were observed surveying more often, indicating that behavioural responses were being utilized to reduce body heat to more normal levels. Surveying can increase body surface area exposed to the environment, which can aid in dissipating heat through non-evaporative heat loss measures including radiation and convection (Whittow et al., 1964; Richards, 1971; Mount, 1979). To the author's knowledge, there has been limited research done examining the effects of thermal stress on the performance of behaviours associated with non-evaporative heat loss.

In addition to surveying, pullets can also increase the amount of body surface area exposed to the environment through being more active and moving away from conspecifics within the crate. Similar to the surveying behaviour, being more active and increasing the space usage of the crate can aid in dissipating heat through non-evaporative heat loss mechanisms

including radiation and convection. Therefore, it is not surprising that pullets in the high T, high RH treatment spent more time active in an attempt to maintain a homeostatic CBT. Few studies have examined activity levels in birds when exposed to hot temperatures, although studies by Richards (1971) noted that the bare skin of the extremities can be a medium by which birds can dissipate heat to the environment during heat exposure. Therefore, by maximizing non-evaporative heat loss, the pullets in this study may have been able to maintain homeostasis during heat exposure. Finally, as a result of increased transport D, pullets were less active, which indicate the pullets have become exhausted by the end of the eight-hour D, or it may also indicate that the pullets have become habituated to the environmental conditions.

2.6 Conclusion

The results from this study indicate that exposure to the various T/RH conditions can influence the CBT of pullets, which can result in behavioural changes and physiological mechanisms being implemented in order to maintain homeostasis. However, implementation of these various mechanisms caused a minor change in the blood physiology of the pullets in this study. Therefore, exposure to the various T/RH combinations in the present study had a minor impact on the welfare of pullets. Additionally, a D of up to eight hours had minimal impacts on the well-being of pullets during simulated transportation at the imposed T/RH conditions. Overall, through changes in behaviour and physiological responses, pullets in the current study were able to cope with the environmental conditions.

3.0 Chapter 3: Effects of simulated transportation conditions on the muscle tissue characteristics of white-feathered layer pullets

Chapter 3 focuses on the impact of exposure to various temperature and humidity combinations for different durations on the muscle tissue characteristics of pullets. Muscle tissue physiology parameters examined in this section include drip loss, thaw loss, cook loss, muscle pH, and muscle colour. In conjunction with the data presented in Chapter 2, the data in this chapter will aid in providing a comprehensive understanding and analysis of the various stress responses exhibited by pullets when exposed to various simulated transport conditions.

Various personnel were involved with this research. As first author, my role was in the collections, analyses, and presentation of the data, and in preparing the following thesis. Kailyn Beaulac played an important role in the preparation and organization of this experiment. Dr. Trever Crowe and, my advisor, Dr. Karen Schwean-Lardner obtained the funding for this study, planned the experimental design, offered essential knowledge, and were editors and reviewers for this thesis. Further review and editing were conducted by my graduate chair, Dr. Fiona Buchanan, and my advisory committee members, Dr. Denise Beaulieu, and Dr. Jennifer Brown.

3.1 Abstract

The objective of this study was to evaluate the effects of various temperature (T) and relative humidity (RH) combinations and exposure durations (D) on the muscle tissue characteristics of layer pullets during simulated transport to use as indicators to assess bird welfare. In a 5x2 factorial arrangement of T and RH, and D the experiment consisted of 3 replications. Pullets (n=240, eight per T/RH combination) were randomly exposed to one of five T/RH combinations (21°C30%RH (21/30), 21°C80%RH (21/80), 30°C30%RH (30/30), 30°C80%RH (30/80), and -15°C (-15)) for a four- or eight-hour D. Before exposure, birds were weighed and placed in one of two environmental chambers. After exposure, final body weights were taken, and then birds were slaughtered using a small-scale facility. After slaughter, carcasses were eviscerated, and an initial pH was obtained from the right breast and thigh. Breast and thigh muscles were removed from carcasses six hours post-slaughter. Final breast and thigh pH and colour values (lightness (L*), yellowness (b*), and redness (a*)) were obtained 30 hours post-slaughter. Left breast muscles were analyzed for thaw and cook loss four weeks post-slaughter. Data were analyzed as a randomized complete block design via an ANOVA (Proc Mixed (SAS 9.4)), with farm of origin as the block. Differences were considered significant at $P \leq 0.05$. Live shrink (kg) was higher for 30/30 and 30/80 compared to 21/80. Breast muscle a* was higher for pullets exposed to 30/30 compared to 21/30. Thigh a* was lower for 21/30 compared to 30/30, and thigh b* was higher for 21/80 compared to -15. With regard to D, live shrink (kg and %) was higher for pullet exposed to the eight-hour D, compared to four-hour D. Breast thaw loss was lower in the four hour D compared to the eight-hour D. Breast b* was higher for pullets exposed for eight-hour D compared to four-hour D. These data indicate that due to heat exposure, and due to increased exposure D, pullets mobilized more energy, resulting in a greater live shrink. An increased transport D had minimal effects on pullet muscle pH, water-holding capacity, and colour.

Keywords: live shrink, thermal stress, muscle pH, muscle colour, cook loss

3.2 Introduction

Though transportation is an essential component of the egg production industry, it can present many stressors (Cheng et al., 2008). One of the most significant stressors for poultry during transport may be the microclimate environment (a combination of temperature (T) and relative humidity (RH)) within the trailer (Knezacek, 2005; Henrikson et al., 2018). The majority of transportation trucks in Canada rely on passive ventilation to dissipate heat and moisture from the trailer (Knezacek, 2005). During passive ventilation, air movement through the trailer is contingent upon a difference in air pressure within and surrounding the trailer, as well as movement of the transport truck, which causes air to enter from the inlets in the rear, and exit through the front of the trailer (Mitchell and Kettlewell, 2009; Knezacek, 2005; Burlinguette et al., 2012). However, due to passive ventilation relying on the movement of the truck, it can be ineffective in circulating and eliminating heat and moisture from the trailer (Knezacek, 2005; Mitchell and Kettlewell, 2009). During transportation in hot ambient T, the curtains will be in an open configuration, which can aid in ventilating the trailer (Knezacek, 2005; Burlinguette et al., 2012). On the other hand, during transportation in cold ambient T, curtains will be in a closed configuration in order to protect the birds on the outside of the trailer; however, this can further reduce the circulation of heat and moisture in the trailer (Knezacek, 2005). In both configurations, T and RH gradients can be created within the trailer, but during the closed configuration, these gradients can be exacerbated, creating high T and high RH conditions directly behind the headboard of the trailer (Knezacek, 2005). These high T and high RH microclimates can be concerning, as it can be challenging for poultry to thermoregulate against these conditions due to both non-evaporative and evaporative heat loss mechanisms becoming ineffective (Freeman, 1984; Lin et al., 2005). The T and RH gradients created during transport can cause thermal stress, which can influence the CBT and physiology of birds, and this may be a welfare concern (Cheng et al., 2008). Additionally, long transportation durations (D) may exacerbate stressors, including poor environmental conditions, resulting in reduced bird welfare (Petracci et al., 2001).

Evaluating the bird's welfare during transport can be difficult, but using several parameters to evaluate what is occurring, behaviourally and physiologically, can make this determination possible. Muscle physiology and muscle tissue characteristics post-slaughter are

good examples. Though pullets are not used for subsequent muscle tissue processing, live shrink, and muscle tissue characteristics including muscle pH, water-holding capacity, and colour have been found in previous research to be an indicator of either heat stress and cold stress during transportation (Dadgar et al., 2010; Dadgar et al., 2012a; Dadgar et al., 2012b). Dadgar et al. (2010) found that the rate of rigor mortis and metabolic and biochemical reactions were influenced by exposure to thermal stress and exhaustion prior to slaughter, which resulted in changes in muscle tissue physiology. Previous research has shown that heat stress pre-slaughter can result in characteristics associated with pale, soft, and exudative (PSE) muscle tissue post-slaughter; whereas exposure to cold stress pre-slaughter results in changes associated with dark, firm, and dry (DFD) muscle tissue post-slaughter (Dadgar et al., 2011; Dadgar et al., 2012a).

Thermoregulation can be energy demanding for poultry, with the extent depending on environmental conditions (Jacobs et al., 2016). Large amounts of energy used to respond to poor environmental conditions can result in high incidences of live shrink during transport, which can be an indicator of poor welfare (Jacobs et al., 2016). Once all readily available glucose in the blood has been used, birds will mobilize energy from fat and protein stores within the body to use as energy and respond to stressors (Sherwood et al., 2013; Jacobs et al., 2016). This is supported by Holm and Fletcher (1997), who observed an increase in live shrink as a result of heat exposure (29°C), and Dadgar et al. (2010), who found that broilers exposed to cold T (-13°C) for three to four hours had a significantly higher live shrink. Additionally, Bianchi et al. (2005) noted that broilers transported for over five hours compared to those transported for less than three and a half hours had a higher live shrink; however, transportation conditions were not mentioned in this study. Nonetheless, this may indicate that as a result of a longer D without feed and water, and potentially the need to thermoregulate against poor conditions for a longer D, a higher live shrink in poultry would be expected (Bianchi et al., 2005).

Glycolysis, which is the enzymatic breakdown of carbohydrates, such as glucose, with the corresponding release of energy and pyruvic acid in muscle, is influenced by heat stress, cold stress, and exhaustion antemortem (Holm and Fletcher, 1997; Dadgar et al., 2012a; Dadgar et al., 2012b). Changes in muscle tissue characteristics are largely dependent on anaerobic glycolysis post-mortem, which is related to the levels of glycogen (the storage form of glucose) present in the muscle post-mortem, as well as the T of the muscle (Honikel, 2004; Dadgar et al., 2011).

Residual glycogen in the muscle post-mortem will undergo anaerobic glycolysis, producing lactate and ATP as products (Honikel, 2004). A build-up of lactate in the muscle will result in a lower muscle pH post-mortem, and high muscle T will accelerate glycolysis leading to a faster build-up of lactate prior to rigor setting in (Pearson and Young, 1989; Honikel, 2004). Previous research has found that broilers exposed to heat stress (29°C) for a 12 hour D had a significantly lower muscle pH compared to those exposed to neutral (18°C) or cold T (7°C) for the same D (Holm and Fletcher, 1997). Additionally, previous research has noted that depending on the specific muscle, and extent of heat stress, muscle pH can decrease to approximately 5.8 before rigor mortis sets in (Honikel, 2004).

An increase in muscle pH post-mortem can indicate cold exposure or fatigue pre-slaughter (Lyon and Buhr, 1999; Dadgar et al., 2010). According to Dadgar et al. (2010), muscle pH will increase as a result of exposure to cold conditions and cold muscle T. Cold muscle T can slow down or cease glycolysis in the muscle, resulting in little to no build-up of lactic acid (Lyon and Buhr, 1999). Furthermore, an increase in muscle pH has been suggested to also be a result of exhaustion ante-mortem and depletion of glycogen stores (Lyon and Buhr, 1999). Thermoregulating against poor environmental conditions can be energy demanding, resulting in birds mobilizing glycogen stores from the muscle for energy (Lyon and Buhr, 1999). Along with this, blood flow ante-mortem will remove any lactate build up in the muscle (Lyon and Buhr, 1999). In many previous studies it was reported that when birds were exposed to T below 0°C for varied D, they had a higher pH compared to those exposed to neutral T; however, these studies did not report an initial pH (Dadgar et al., 2010; Dadgar et al., 2011; Dadgar et al., 2012b). Due to the effect of muscle T and levels of residual glycogen on muscle pH, this can be a useful indicator of thermal stress and fatigue in poultry pre-slaughter.

Changes in muscle drip, thaw, and cook loss have been found to coincide with changes in the muscle pH (Pearson and Young, 1989; Honikel, 2004). Honikel and Hamm (1994) and Pearson and Young (1989) noted that with decreasing muscle pH, the protein filaments will reach their isoelectric point (pH at which the protein has no electric charge), causing an increased attraction between the amino acid side chains, resulting in less space between the filaments. As this space decreases, water is no longer immobilized and can be lost (Pearson and Young, 1989; Honikel and Hamm, 1994). As mentioned previously, heat exposure can cause a decrease in

muscle pH, which would therefore result in a higher drip, thaw, and cook loss due to less space between protein filament, which is in accordance with Dadgar et al. (2010) who found a higher thaw and cook loss in the breast muscles of broilers exposed to temperatures between 20°C and 30°C (three hour D).

Exposure to cold T, and/or exhaustion antemortem, resulting in an increase in muscle pH, can cause a decrease in muscle drip, thaw, and cook loss by counteracting the physiological mechanisms described above, leading to more bound water in the muscle (Sandercock et al., 2001; Honikel, 2004). Dadgar et al. (2012a) observed a lower cook and thaw loss in the breast muscles of broilers exposed to -14°C for three hours prior to slaughter. Dadgar et al. (2011) exposed broilers to T below -14°C for three hours, and found a lower thaw and cook loss, but also observed a higher drip loss in the muscle of birds exposed to cold T pre-slaughter. Not all research agrees, however, as Babji et al. (1982) found no effect of cold exposure (4°C) on either broiler muscle thaw loss or cook loss. Differences in results between previous research and that of the current study can be due to different exposure conditions, exposure D, and differences in species used for the various studies.

Previous studies have shown that exposure to heat or cold stress prior to slaughter can cause changes in muscle tissue colour post-mortem (Dadgar et al., 2012a; Dadgar et al., 2012b). Muscle colour has been suggested to be highly correlated with muscle pH, as a high pH will result in a darker colour, and a lower pH will result in a paler colour (Pearson, 1994; Fletcher, 1999). As indicated previously, muscle pH can be influenced by exposure to either heat or cold stress, or fatigue pre-slaughter, therefore changes in muscle tissue colour may also be indicative of heat stress, cold stress, or exhaustion ante-mortem (Pearson, 1994; Dadgar et al., 2010). Muscle tissue colour is often quantified by the "LAB" scale (lightness (L*), redness (a*), and yellowness (b*)) (Bianchi et al., 2006). Rapid declines in muscle pH caused by exposure to heat stress can result in rapid denaturation and precipitation of muscle tissue proteins, producing a pale colour (high L*, low a* and b*) (Pearson and Young, 1989; Pearson, 1994; Bianchi et al., 2006). However, results from previous research examining changes in muscle colour in birds that have been subjected to heat stress pre-slaughter are inconsistent. For example, Holm and Fletcher (1997) and Petracci et al. (2001) found that there were no changes in the L* values, and

conflicting results in both the a* and b* values of the breast muscles of broilers exposed to temperatures above 29°C for 12 hours.

Cold exposure can also influence muscle tissue colour. Studies conducted by Dadgar et al. (2011) and Dadgar et al. (2012a) reported that when birds were exposed to cold T (below -14°C), lower L* values and higher a* values were noted, which are indicators of darker muscle tissue. A darker muscle tissue colour due to an elevated pH can be produced through various mechanisms (Pearson, 1994). A muscle that has a high pH will tend to have a higher concentration of myoglobin and hemoglobin, which would cause the muscle to appear darker (Fletcher, 2002). Additionally, when this muscle tissue is exposed to air, the oxymyoglobin will go through reduction reactions forming metmyoglobin and deoxymyoglobin which have a predominantly brown or purple colour, respectively (lower L*, higher a* and b*) (Boulianne and King, 1998; Mancini and Hunt, 2005; Suman and Joseph, 2013). Lastly, the darker colour may also be caused through a higher water-binding capacity leading to higher translucence and absorption of light (Pearson, 1994).

Previous research has observed an impact of exposure to either heat stress or cold stress pre-slaughter on the muscle pH, water-holding capacity, and colour. However, increased journey D may also present a concern for the well-being of birds during transportation. Exposure to poor environmental conditions for a longer period of time may exacerbate the resultant changes in muscle tissue characteristics post-slaughter (Zhang et al., 2009). Little research has been conducted observing the effects of increased exposure D on these muscle tissue characteristics. Therefore, it is not well known how the muscle pH, water-holding capacity, and colour would change as a result of an increased transportation D.

Egg production pullets are transported to a laying facility at approximately 17 weeks of age (NFACC, 2016). Despite many studies being conducted on the transportation of broilers, very little is known regarding the transportation of pullets, and pullet stress responses during transportation. Additionally, little is known regarding the effect of increased transportation D on stress responses and the well-being of pullets. The objective of this chapter was to compare how exposure to hot and neutral T, with a high or low RH, as well as to a cold T during simulated transportation for various D, would influence pullet live shrink and muscle tissue characteristics, which were used to asses bird welfare. Parameters examined were live shrink, muscle pH, drip

loss, thaw loss, cook loss, and muscle tissue colour. The overall hypothesis was that exposure to either heat stress or cold stress during simulated transportation would require pullets to thermoregulate, which would result in changes to live shrink and muscle tissue characteristics. Specifically, it was hypothesized that exposure to hot T, with high or low RH, compared to the neutral conditions, would result in a higher live shrink due to the energy demands of thermoregulation. Additionally, it would result in characteristics associated with PSE muscle tissue due to decreased muscle pH. Exposure to cold conditions, compared to the neutral conditions, would also result in a higher live shrink due to the energy demands of thermoregulation, as well as it would result in characteristics associated with DFD muscle tissue due to an increase in the muscle pH. Finally, it was hypothesized that as a result of increased exposure D, changes in live shrink and muscle tissue characteristics would be exacerbated due to pullets utilizing thermoregulation techniques against the poor environmental conditions for a longer period of time.

3.3 Materials and Methods

The experimental protocol for this research was approved by the University of Saskatchewan Animal Care Committee and was performed under the guidelines of the Canadian Council of Animal Care (1993, 2009).

3.3.1 Experimental Design

The impacts of exposing white-feathered egg production pullets to various T and RH combinations for one of two D on bird welfare were evaluated through exposing birds to one of five T/RH combinations (30°C with 80% RH (30/80), 30°C with 30% RH (30/30), 21°C with 80% RH (21/80), 21°C with 30% RH (21/30), and -15°C with uncontrolled humidity (-15)). Each group of T/RH combination birds was exposed to the conditions for either a four- or eight-hour D.

A total of 240 white-feathered commercial layer pullets were used and included three replicate trials (80 pullets per trial). Pullets (18-19 weeks of age) used for each replication were from a separate commercial flock. For this study, 16 pullets (eight pullets per D) were exposed to each T/RH conditions across both the time D. For each T/RH replicate, two transport crates were used (one crate per time D), containing eight pullets per crate at a stocking density of 45.5 kg/m^2.

Two treatments were performed over one night for a total of three nights per each replicate (see Figure 2.1).

Two environmental simulation chambers (College of Engineering, University of Saskatchewan, Saskatoon, Saskatchewan; Figure 2.1) were used to create five different T/RH conditions. The RH within one chamber was controlled by a steam generator (Nortec, H.V.A.C Sales (1995) LTD) which was controlled via a computer software system (LabVIEW), and the RH in the second chamber was controlled by using either one or two humidifiers (Essick Air Products Evaporative Humidifier), or a dehumidifier (NOMA, dehumidifier), as necessary. Systems controlling chamber T and RH were turned on two hours before the pre-treatment procedures to allow chambers to stabilize to their respective conditions. Afterwards, two identical crates were labelled with the respective T/RH and time D and equipped with a T and RH data logger (DS1923, Maxim iButton) on the left side of the crate wall, at bird level. Data loggers recorded chamber T and RH every 60 seconds while birds were being exposed to respective T/RH conditions. Crates were then placed on tables inside of chambers and equipped with a removable lid made of chicken wire and a wood frame (see Figure 2.2). A humidity sensor was attached to the side of each chamber to monitor RH in real-time and was controlled by the same system as the steam generator. A thermocouple attached to a multimeter (Omega HH509, Omega Engineering Inc., Laval, Quebec, Canada) was also attached to the side of each chamber to monitor chamber T in real-time. Prior to the commencement of the pre-treatment procedures, the lights within the chambers were turned off.

3.3.2 Birds and Housing

White-feathered egg production pullets were purchased from commercial farms within a 250-kilometer radius of Saskatoon, Saskatchewan, and were transported to a livestock facility at the University of Saskatchewan. Pullets were transported in one trip via an enclosed van. Information regarding farm of origin, bird strain, and age are indicated in Table 2.1. The birds were given a three-day acclimation period before being exposed to the T/RH conditions. A floor pen (389 x 300 cm) containing straw litter inside the livestock facility was used to house the pullets. Pens were cleaned between each flock, and new straw was laid. Water was provided ab libitum via two bell drinkers (38 cm diameter). Feed was purchased from the producer when picking up pullets and was provided ab libitum via three tube feeders (36 cm diameter).

Information on lighting program and barn set T were obtained from the farm of origin and implemented inside the livestock housing facility on campus. Birds were checked twice daily and feeders where filled when necessary. During the acclimation period, no mortality occurred. Pullets were not feed restricted prior to simulated transportation.

3.3.3 Pre-Treatment Procedure

On collection day, pullets (16 per T/RH and D, eight per D) were randomly assigned to one of the five T/RH treatments. An initial individual body weight was recorded for each pullet. Afterwards, pullets from each T/RH combination were wing banded (two bands per bird) for identification. From the extreme treatments only (30/80 and -15), four birds per T/RH combination (two per time D) were administered a transponder (IPTT-300, Bio Medic Data Systems, Delaware, US) subcutaneously along the body, beneath the right wing. These birds were coloured with a livestock marker for identification. Transponders were used to monitor the surface body T of the pullets while inside the chambers, and this was used to help identify a humane endpoint for the pullets under distress (see section 3.3.5). Once all birds for the T/RH combination across both time D were prepared and crated, birds remained in lairage for a 15-minute period. After the lairage period, both crates containing the pullets were transported one and a half kilometers to the building containing the environmental simulation chambers. Birds were transported in one trip via an enclosed van. Following arrival, crates were placed on a rolling cart and transported to the room containing the environmental simulation chambers.

3.3.4 Treatment Procedure

Once pullets arrived in the room containing the environmental simulation chambers, they were placed into the respective predetermined time D crate inside the chamber with the appropriate T/RH conditions. During exposure, lights within the chambers remained off. Crates were taken in and out of the chambers according to the schedule presented in Figure 2.1. In extreme T/RH conditions (30/80 and -15), a transponder reading was recorded using a transponder wand (BMDS IPTT DAD-8007-IUS) every 30 minutes while birds from all other T/RH conditions and D were disturbed (sham treatment) every 30 minutes by placing a hand on each bird in the crate and gently agitating the bird. Crates were removed from the chamber after the respective exposure D.

3.3.5 Humane Intervention

Several humane intervention points were identified and implemented to reduce bird suffering while they were being monitored throughout the experiment. Pullets displaying behaviours indicating heat or cold stress (panting, shivering, or ptiloerection) were not removed from this study, as one of the objectives of this study was to observe and understand how the environmental conditions influenced these behaviours. At the same time as transponder readings were being taken, a trained individual observed the pullets in both crates, using a small headlamp, for indicators of any bird appearing incapable of coping with the exposure conditions, such as displaying distress behaviours. If any bird was perceived as being in distress, as seen by displaying excessive heat or cold stress behaviours (panting, shivering, or torpor (temporary state of dormancy)), as determined by the trained individual, the bird was removed from the study. If transponder readings were found to be approximately 43°C, birds were monitored every 15 minutes for indicators of distress (outlined above). Additionally, if transponder readings were found to be below approximately 37°C birds were monitored every 15 minutes for indicators of distress (outlined above). The end of this experiment was designated as removal from the environmental chambers after exposure. No birds were removed as a result of humane intervention.

3.3.6 Post-treatment Procedure

At the end of the respective exposure D, the T and RH data logger was removed from the crate, and the data were downloaded onto a computer software system for further analysis (1-Wire).

With respect to the birds, final individual body weights were taken for all birds. Thereafter, birds with identifying coloured wings (transponder birds) were euthanized via cervical dislocation by a trained individual. Transponders were then removed, and carcasses were placed in a bin for disposal. All remaining pullets were then shackled and stunned for 30 seconds using an electric knife (VS200, Midwest Processing Systems, Minneapolis, MN, USA), at power level six (circa 0.16 amps, 60 Hz AC), or until the wing drop response and the absence of the nictitating response was noted (Henrikson et al., 2018). Five carcasses per time T/RH and time D were then further processed, while the remaining carcasses were placed in a bin for disposal. The five further processing carcasses were then scalded (65-68°C) using a commercial

scalder (Chicken Dipper Scalding Tank DUX, 120 volts), mechanically plucked (Featherman Feather Picker K7080, 10.8 Amps), and eviscerated.

After evisceration, an initial pH reading was taken from both the right pectoralis and right iliotibial muscles from the five carcasses. Afterwards, the whole carcasses were then chilled in an ice water bath for one hour and then chilled on ice for five hours. Six hours post-slaughter, the carcasses were taken off ice, and the right pectoralis and iliotibial muscles were removed. The right breast and thigh muscles would then be used for further analyses (30 hours post-slaughter) of muscle ultimate pH, drip loss, and colour. The left pectoralis muscles were also removed from the carcasses and used for analyses of muscle thaw loss and cook loss. Wing bands were removed from all carcasses before placement in bins for proper disposal.

3.3.7 Data Collection

Figure 3.1 outlines the experimental collection procedures and the data measurements recorded. For each time period presented in Figure 3.1, the colour of the procedure conducted corresponds with the respective measurement taken from the procedure. Collections procedures were performed as outlined for all three replications.

Live Shrink: Individual weights were obtained using a digital hanging scale (50# digital scale, Berkley, Columbia, SC). Weigh scale was calibrated using a one kilogram weight before pullet weights were taken both pre- and post-treatment. Live shrink (%) was calculated by subtracting the final body weight from the initial body weight, dividing by the initial body weight, and multiplying by 100% to obtain a final value represented as a percent.

Breast and Thigh pH: Initial pH readings were taken from a vertical incision made in pectoralis or iliotibial muscle using a scalpel. Final pH readings were taken from a second vertical incision made adjacent to the initial. Initial and final pH readings were taken from the vertical incisions using a pH probe (Accumet, Fisher Scientific, Ottawa, ON, Canada) with a pH meter and T probe (Hanna H1 9025 microcomputer pH meter, Woonsocket, Rhode Island, US).

Breast and Thigh Drip Loss: Individual weights were obtained on both the right breast and thigh muscles using a scale (Navigator^{TM} Ohaus, Ohaus Corporation), immediately after removal from the carcass. Both muscles were then placed on a labelled Styrofoam tray, wrapped with plastic wrap, and placed in a refrigerator for 24 hours. After refrigeration, final individual

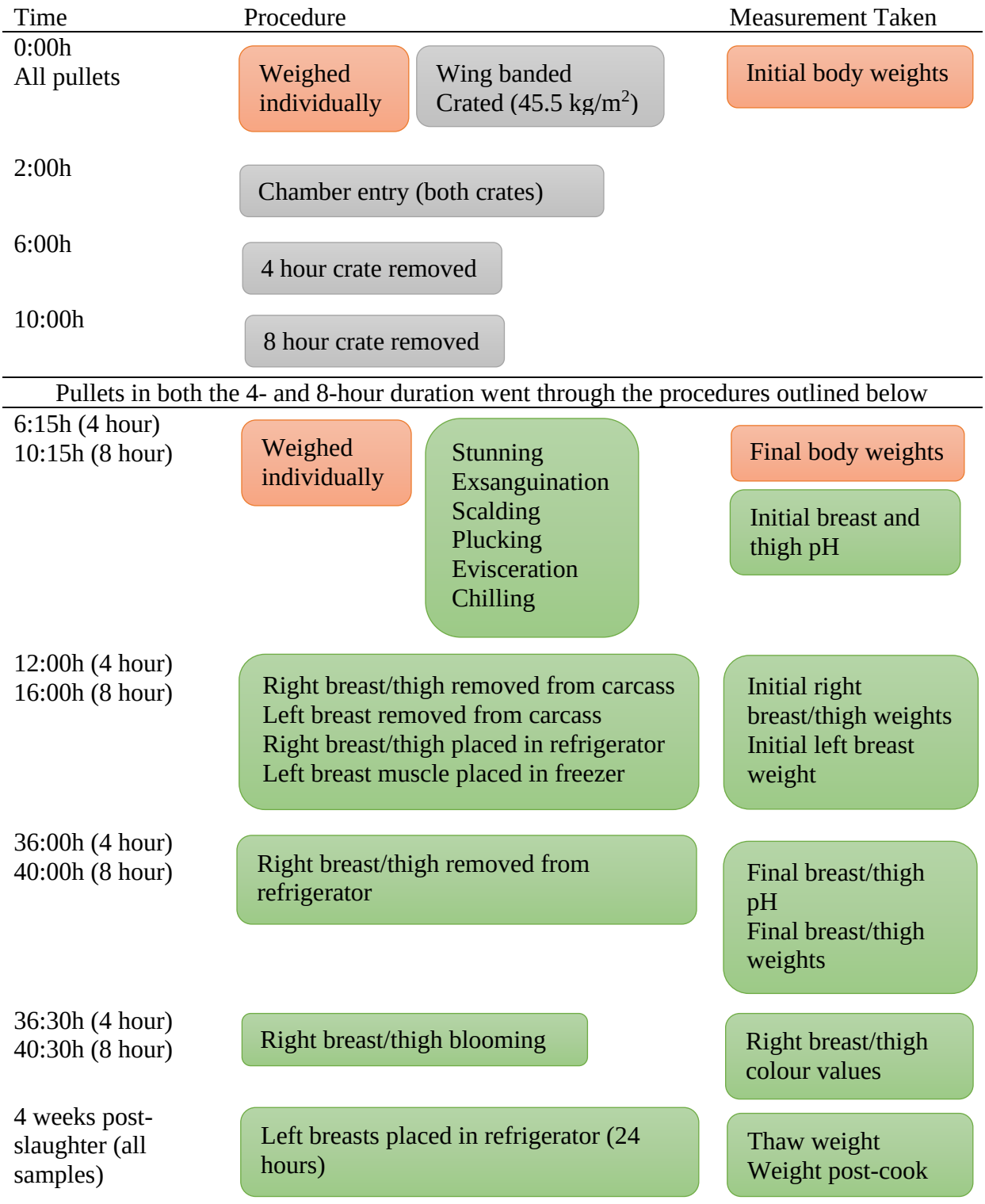

Figure 3. 1. Experimental procedures and data measurements collected (modified from Henrikson, 2017).

weights of both muscles were obtained. To calculate drip loss (%), the initial weight was subtracted from the final weight, then divided by the initial weight, and multiplied by 100% in order to obtain a final value for drip loss, which was represented as a percent.

Breast and Thigh Colour: After the final pH readings were recorded, the inner part of the pectoralis major and minor muscles, and the right iliotibial muscle were left exposed to air for 30 minutes (blooming). After blooming, two colour readings were obtained from the breast and thigh muscles using a Minolta colour meter (CR-400, Konica Minolta Sensing Americas, Ramsey, NJ, US). The second colour reading was taken with the device rotated 90 degrees after the first reading to account for the difference in muscle fiber orientation. Readings on the lightness, redness, and yellowness values, L*, a*, and b*, respectively, were obtained.

Breast Thaw Loss and Cook Loss: After removal from the carcass the left pectoralis muscles were weighed using a scale (Navigator^{TM} Ohaus, Ohaus Corporation), placed in a labelled Ziploc bag, and then placed in a freezer for four weeks until further analyses. Upon removal from the freezer, the left breast muscles were placed in a refrigerator to thaw for 24 hours, then were individually removed from the Ziploc bag, blotted with a paper towel, and the individual post thaw weights were obtained using a scale. Breast samples were then placed in a labelled Ziploc bag. Samples were prepared in groups of five. In one sample per group, a small incision was made in the breast, and a thermocouple, attached to a multimeter (Omega HH509, Omega Engineering Inc., Laval, Quebec, Canada), was placed inside the incision to monitor the T of the breast sample while cooking. Sample groups were then placed in a hot water bath (Isotemp, Model 2340) at 80°C, and left until the internal T of the sample containing the thermocouple reached 75°C. At that point, samples were left to cook for an additional five minutes, then were taken out of the hot water bath and individual post-cooking T was taken using a meat thermometer. Samples were left to cool until the internal T of the samples were between 40-50°C (determined using the meat thermometer). After cooling, samples were taken out of the Ziploc bag, blotted with a paper towel, and individual final post-cooking weights were taken. Thaw loss (%) was determined by subtracting the initial weight from the thaw weight, dividing by the initial weight, and multiplying by 100% to obtain a final value for thaw loss. Cook loss (%) was determined by taking the final, post cook weight, subtracting the initial

weight, dividing by the initial weight, and multiplying by 100% to obtain a final value for cook loss.

3.3.8 Statistical Analyses

The experiment was analyzed as a 5x2 factorial arrangement (T/RH and D) in a randomized complete block design (RCBD). The experimental block was farm of origin, and the experimental unit was crate. There were three replicates per T/RH, and D. Data were checked for normality using Proc Univariate prior to analyses, and data were log transformed when ANOVA assumptions were not met. An ANOVA via a Proc Mixed procedure (SAS® 9.4, Cary, NC) was used to analyze data, with means separation conducted using the Tukey test. Significant differences were considered at $P \leq 0.05$ and trends were noted when $0.05 < P \leq 0.10$.

3.4 Results

Live Shrink: Live shrink (kg) was affected by the T/RH combinations with pullets in the 30/30 and 30/80 conditions having a higher live shrink, compared to pullets in the 21/80 treatment (Table 3.1). However, when live shrink was converted to a percentage, only a tendency towards an impact of T/RH combination on pullet live shrink was noted. A trend towards significance (P=0.056) was noted for the final body weights of pullets as a result of the T/RH. Pullets exposed to the conditions for eight hours had a significantly higher live shrink (kg and %) compared to pullets exposed to conditions for four hours (Table 3.1).

Drip, thaw, and cook loss: The right breast muscles from pullets exposed to the 21/30 and 21/80 conditions had a heavier initial and final weight, compared to those exposed to the 30/30 condition (Table 3.2). There was no significant effect of T/RH combinations on either right breast or thigh thaw, cook, or drip loss (Table 3.2 - Table 3.4); however, there was a trend towards significance on the left breast initial weight (P=0.091), final weight (P=0.068), and thaw loss (P=0.081) (Table 3.2). With regards to D, breast thaw loss (kg and %) was greater for pullets exposed for four hours compared to those exposed for eight hours (Table 3.2). With regards to exposure D, there was a trend towards significance for breast cook loss (P=0.061). There was no effect of D on breast or thigh muscle drip loss.

Muscle pH: There was no interaction effects between the T/RH combinations and D on pullet muscle tissue pH. There was no impact of either T/RH combinations or D on the pH of the

Table 3. 1. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (n=15), on the live shrink of white-feathered layer pullets.

	T/RH Combinations						D				
Parameter	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[2]
Initial Body Weight (kg)	1.29	1.29	1.29	1.26	1.29	0.342	1.28	1.29	0.193	0.180	0.011
Final Body Weight (kg)	1.26	1.27	1.27	1.22	1.24	0.056	1.25	1.25	0.824	0.250	0.010
Live Shrink (kg)	**0.03^{ab}**	**0.02^{ab}**	**0.02^{b}**	**0.04^{a}**	**0.05^{a}**	**0.035**	**0.03^{b}**	**0.04^{a}**	**0.004**	0.903	0.004
Live Shrink (%)	2.44	2.02	1.44	3.28	3.47	0.066	**1.87^{b}**	**3.20^{a}**	**0.011**	0.910	0.295

[1]Treatment at uncontrolled humidity

[2] Pooled standard error of mean

-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

Table 3. 2. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (n=15), on the thaw loss and cook loss of the left breast muscle of white-feathered layer pullets.

	T/RH Combinations						D				
Parameter	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[2]
Left Breast											
Initial Breast Weight (g)	74.76	80.60	73.77	73.37	75.53	**0.091**	76.37	74.84	0.380	0.797	3.497
Weight Post Thaw (g)	71.73	77.66	70.71	70.96	72.96	**0.068**	73.29	72.32	0.543	0.731	3.316
Final Breast Weight (g)	58.49	63.18	57.29	57.69	59.43	0.104	59.27	59.17	0.941	0.822	2.848
Thaw loss (g)	-3.03	-2.94	-3.06	-2.41	-2.57	0.245	**-3.08[b]**	**-2.52[a]**	**0.019**	0.802	0.227
Thaw Loss (%)	-4.07	-3.42	-4.23	-3.14	-3.35	**0.081**	**-4.04[b]**	**-3.25[a]**	**0.010**	0.685	0.196
Cook Loss (g)	-13.23	-14.48	-13.42	-13.27	-13.53	0.858	-14.02	-13.15	0.307	0.230	0.612
Cook Loss (%)	-24.63	-23.21	-24.61	-23.47	-23.25	0.936	-24.56	-23.11	**0.061**	0.250	0.775

[1]Treatment at uncontrolled humidity

[2] Pooled standard error of mean

Delta (Δ) = final – initial

-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

Table 3. 3. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (n=15), on muscle tissue characteristics of the right breast muscle of white-feathered layer pullets.

Parameter	T/RH Combinations					*P*-Value	D		*P*-Value	Interaction *P*-Value	SEM[2]
	-15	21/30	21/80	30/30	30/80		4 hours	8 hours			
Right Breast											
Initial Breast Weight (g)	**76.43^{ab}**	**80.47^{a}**	**81.83^{a}**	**71.85^{b}**	**78.18^{ab}**	**0.008**	78.34	77.16	0.468	0.531	1.643
Final Breast Weight (g)	**75.87^{ab}**	**79.89^{a}**	**81.04^{a}**	**71.22^{b}**	**77.71^{ab}**	**0.008**	77.72	76.58	0.479	0.583	1.700
Drip Loss (g)	-0.56	-0.58	-0.79	-0.63	-0.47	0.136	-0.62	-0.58	0.126	0.292	0.098
Drip Loss (%)	-0.73	-0.77	-1.00	-0.98	-0.64	0.469	-0.84	-0.81	0.934	0.417	0.154
Initial pH	6.64	6.51	6.58	6.59	6.63	0.607	6.59	6.59	0.969	0.321	0.029
Final pH	5.70	5.83	5.68	5.70	5.74	0.339	5.71	5.75	0.454	0.957	0.028
Δ pH	-0.94	-0.68	-0.90	-0.89	-0.89	0.290	-0.88	-0.84	0.664	0.638	0.047
L*[3]	50.06	48.95	49.31	49.22	49.73	0.794	49.96	48.95	0.128	0.653	0.287
a*[3]	**5.31^{ab}**	**4.94^{b}**	**5.44^{ab}**	**6.09^{a}**	**5.97^{ab}**	**0.024**	5.47	5.64	0.409	0.281	0.134
b*[3]	1.25	0.79	0.92	0.96	1.05	0.652	**1.22^{a}**	**0.76^{b}**	**0.038**	0.331	0.150

[1] Treatment at uncontrolled humidity
[2] Pooled standard error of mean
[3] Muscle tissue colour, n=4 for T/RH, n=10 for D
L* = Lightness
a* = Redness
b* = Yellowness
Delta (Δ) = final – initial
-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

Table 3. 4. Effect of various temperature (T) and relative humidity (RH) combinations (n=6), and two different time durations (n=15), on the muscle tissue characteristics of the thigh muscle of white-feathered layer pullets.

	T/RH Combinations						Duration				
Parameter	-15	21/30	21/80	30/30	30/80	*P*-Value	4 hours	8 hours	*P*-Value	Interaction *P*-Value	SEM[2]
Right Thigh											
Initial Thigh Weight (g)	61.03	60.91	61.43	58.48	59.23	0.333	60.47	59.96	0.624	0.495	0.545
Final Thigh Weight (g)	60.59	60.62	60.95	57.97	58.84	0.293	60.03	59.55	0.643	0.423	0.569
Drip Loss (g)	-0.44	-0.29	-0.48	-0.51	-0.39	0.132	-0.44	-0.41	0.475	0.236	0.071
Drip Loss (%)	-0.75	-0.49	-0.78	-0.85	-0.67	0.121	-0.74	-0.67	0.444	0.171	0.120
Initial pH	6.39	6.38	6.21	6.33	6.32	0.280	6.33	6.32	0.971	0.389	0.040
Final pH	6.16	6.28	6.05	5.95	6.13	0.151	6.09	6.13	0.629	0.873	0.053
Δ pH	-0.23	-0.10	-0.16	-0.38	-0.19	0.305	-0.24	-0.19	0.619	0.919	0.057
L*[3]	49.84	51.73	50.72	50.07	50.47	0.157	50.59	50.54	0.922	0.821	0.257
a*[3]	**4.95^{ab}**	**4.16^{b}**	**4.43^{ab}**	**5.43^{a}**	**4.85^{ab}**	**0.019**	4.93	4.60	0.122	0.396	0.173
b*[3]	**-3.85^{b}**	**-2.56^{ab}**	**-2.07^{a}**	**-2.85^{ab}**	**-2.96^{ab}**	**0.048**	-2.68	-3.03	0.274	0.609	0.207

[1] Treatment at uncontrolled humidity
[2] Pooled standard error of mean
[3] Muscle tissue colour n=4 for T/RH, n=10 for D
L* = Lightness
a* = Redness
b* = Yellowness
Delta (Δ) = final – initial
-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

breast muscle (Table 3.3). Additionally, there was no impact of either the T/RH combinations or D on the pH of thigh muscle (Table 3.4)

Muscle colour: Neither breast or thigh lightness (L*) was affected by T/RH conditions or D of exposure. The redness of the breast and thigh were significantly impacted by the T/RH conditions. For the right breast, redness (a*) was higher in the 30/30 treatment compared to the 21/30 treatment (Table 3.3). Thigh muscle a* followed the same pattern as with the breast, with a* being higher in the 30/30 treatment, compared to the 21/30 treatment (Table 3.4). Thigh muscle yellowness (b*), compared to the 21/80 treatment, was lower in the -15 treatment (Table 3.4). An impact of D was only noted on the b* of the breast muscle from pullets exposed for four hours being higher than those exposed for the eight hour D.

3.5 Discussion

Implementing thermoregulatory and physiological mechanisms during exposure to either hot or cold T can be energetically costly (Holm and Fletcher, 1997). Live weight loss can be a useful measure to indicate the amount of energy mobilized and used during transportation (Holm and Fletcher, 1997; NFACC, 2018). Large losses in live weight during transportation can, therefore, indicate that birds are expending large amounts of energy, which may be a welfare concern. The data from the current study may indicate that, compared to the pullets in the 21/80 treatment, pullets in both of the hot T treatments mobilized and expended more energy in order to respond to the heat stress. These results agree with previous research by Holm and Fletcher (1997) and Petracci et al. (2001) who found a 6.29% and a 5.67% decrease in the live weight, respectively, of broilers exposed to T above 29°C for 12 hours. In both of the previously mentioned studies, the broilers were feed restricted for 12 hours, as opposed to the pullets in the current study who were not feed restricted. In addition to feed restriction, a higher loss in live weight in the mentioned studies, as opposed to the current study, could also be a result of species differences between broilers and layer pullets. However, both the current study and previous research indicate that thermoregulation to control the effects of heat stress can be energy-demanding as shown by higher losses in live weight, which may be a concern for the well-being of birds during transport.

An effect of transport D on live shrink was noted in the current study, with pullets exposed for the eight-hour D having a higher live shrink compared to those exposed for the four

hour D. This is in accordance with the results presented by Bianchi et al. (2005) who found a higher live shrink (2.09%) in broilers transported for over five hours, compared to those transported for less than five hours; however, transportation conditions and feed restriction D prior to transport were not mentioned. Nonetheless, due to increased transportation D, pullets mobilized more energy in order to respond to stressors present during transport, resulting in an increased loss in live weight. Additionally, pullets exposed for the eight hour D were without feed and water for a longer D, which resulted in a higher live shrink.

Changes in muscle pH associated with either PSE or DFD muscle tissue can be related to exposure to hot or cold T prior to slaughter, respectively (Zhang et al., 2009; Dadgar et al., 2010; Dadgar et al., 2012a). Muscle tissue that can be classified as PSE typically has an ultimate pH (24 hours post-slaughter) of approximately 5.7 or lower, whereas muscle tissue classified as DFD has an ultimate pH of approximately 6.1 or higher, however, this can vary depending on the muscle type (Dadgar et al., 2010; Dadgar et al., 2011). In the current study, there was no effect of either the T/RH treatments, or D on breast or thigh muscle pH. However, the ultimate pH of the breast muscle ranged from 5.68 to 5.83, which may be classified as PSE, whereas the ultimate pH of the thigh ranged from 5.95 to 6.28, which is suggestive of DFD muscle tissue. Interesting to note in the current study, regardless of the T/RH exposure conditions, or exposure D, the final pH for both the breast and the thigh muscles decreased compared to the initial pH reading, though no differences were significant. The results of the present study differ from that found by Dadgar et al. (2012a) who observed a higher breast muscle pH of broilers (five to six weeks of age) exposed to cold temperatures (below -9°C) for three hours, as opposed to broilers in the control group (20°C exposure for three hours). However, the mentioned study used core samples to determine breast muscle pH, which may be more accurate compared to using a pH probe. With regards to heat exposure, Holm and Fletcher (1997) observed a lower pH in the breast muscle of broilers exposed to 29°C for 12 hours pre-slaughter, compared to those exposed to T below 18°C for the same D. Dadgar et al. (2010) subjected broilers to 25°C with 46% RH for a three-hour D and observed a significant decrease in the ultimate pH post-slaughter. The mentioned study determined the ultimate pH using a slurry method, which may have provided more accurate results compared to using a pH probe. An initial pH was not indicated in the previously mentioned studies, though the ultimate pH was found to be consistent with that of the breast muscle in the current study. In the current study, no effect of treatment exposure on

muscle pH may be due to birds having to use energy stores to cope to stressors during transport; therefore, only small amounts of glycogen would be left in the muscle for post-mortem glycolysis (Lyon and Buhr, 1999). However, no significant changes in muscle pH resulting from T/RH conditions could also indicate that pullets were able to implement mechanisms that were effective in maintaining a homeostatic body T, resulting in no changes in muscle tissue characteristics.

Drip loss, thaw loss, and cook loss were not affected by T/RH exposure conditions. These factors, which are determinates of the muscle water-binding capacity, are directly correlated with the muscle pH (Pearson and Young, 1989). With no effects seen in either the breast or thigh ultimate pH, it would be expected that no significant differences in the drip, thaw, or cook loss of either the breast or thigh muscles would be noted. Significant differences were noted in the initial and final weights for the right breast as a result of T/RH exposure; however, when drip loss for the right breast was calculated, differences were no longer significant. Dadgar et al. (2010) also found no effects on the drip loss of birds exposed to either hot (25°C, 46%RH), or cold (-7°C, 76%RH) conditions. Although significant differences in cook loss of the breast were observed, the authors noted that these differences did not follow any specific trend (Dadgar et al., 2010). Holm and Fletcher (1997) found a lower cook loss in broilers exposed to 29°C for 12 hours, compared to those exposed to either 18°C or 7°C for the same D. Another study by Babji et al. (1982) exposed turkey toms (26 weeks old) to neutral (21°C), hot (38°C), and cold (4°C), conditions for four hours, and did not find any significant differences in either breast thaw loss or cook loss. Dadgar et al. (2011) found a significantly lower thaw and cook loss in broilers exposed to temperatures below 0°C, but found a higher drip loss in birds exposed to temperatures below -14°C. Differences in results observed in these studies, as opposed to the current study, may have been due to a number of factors including species differences, differences in exposure conditions and D, as well as no initial or final weights being indicated in any of the previously mentioned studies.

An effect of D was observed on thaw loss, with breast muscles from pullets exposed for a four-hour D having a greater thaw loss (g and %) compared to those exposed for eight hours. Thaw loss can be correlated with muscle pH, but no impacts of exposure D were found for muscle pH. Additionally, no effects were noted for either drip loss or cook loss. Little is known

regarding the effects of transport D on muscle thaw loss, independent of transport conditions. Therefore, it is difficult to draw conclusions on the effect of transportation D on the thaw loss of the breast muscle tissue.

According to previous research, PSE and DFD muscle tissue are primarily determined based on an L* value of ≥53 and ≤46, respectively (Dadgar et al., 2012a; Dadgar et al., 2012b). Previous studies exposing broilers to cold T (below -13°C) have found L* values consistent with DFD muscle tissue (Dadgar et al., 2011; Dadgar et al., 2012a); however, studies exposing broilers to hot T (above 29°C) resulted in no effects on breast muscle L* values (Holm and Fletcher, 1997), though exposure D varied for all of the previously mentioned studies. In the current study, T/RH combinations did not result in any effect on the L* values of either the breast or thigh muscle. Additionally, inconsistent T/RH effects were found on muscle colour with the breast and thigh muscle having a higher a* in the 30/30 and a lower a* in the 21/30, and the thigh muscle having a higher b* in the 21/30 treatment and a lower b* in the -15 treatment. Changes in muscle tissue colour are closely correlated with the muscle pH, but the results found for muscle colour in the current study do not follow the same pattern as found for muscle pH. No significant differences were found for breast or thigh pH with regards to the various T/RH combinations, though, compared to the initial pH, the ultimate pH regardless of T/RH exposure conditions was more acidic. A lower pH would result in denaturation of proteins in the muscle, causing a lighter muscle colour (higher L*) (Pearson, 1994). This was not found in a study by Vermette et al. (2017) who observed a lower pH in turkey hens (12 weeks of age) exposed to 35°C, as opposed to hens exposed to 20°C, but saw no effect on muscle a* or b*. However, in the mentioned study, colour readings were taken from an incision in the muscle 27 hours post-mortem, as opposed to the current study, in which colour readings being taken from the surface of the muscle 30 hours post-slaughter (Vermette et al., 2017). Lastly, pullets exposed for the eight-hour D had a lower breast muscle b*, compared to those exposed for the four hour D; however, little is known regarding the impact of transportation D on pullet muscle colour. The results for muscle colour in the current study may be confounded as colour values were only obtained for two replications.

3.6 Conclusion

The results from this study indicate that thermoregulatory mechanisms can be metabolically demanding, and birds exposed to heat stress must use more energy to thermoregulate and cope with the environmental conditions. This can, therefore, result in increases in live shrink, which can negatively impact pullet welfare. Additionally, longer transport D can negatively influence welfare through long D without feed and water, and exacerbated poor environmental conditions, leading to larger live weight losses.

The results from the current study found minimal and inconsistent changes in the muscle tissue characteristics of the pullets. These minimal changes suggest that birds were able to maintain homeostasis in the respective environments through the implementation of behavioural and physiological mechanisms. It may also suggest that the birds used up their glycogen stores ante-mortem, therefore there were little stores left to go through glycolysis post-mortem; however, this cannot be confirmed.

Overall, the results of this study support the hypothesis that live shrink will increase as a result of heat exposure and longer transportation D. However, the results in this study do not support the hypotheses that indicate muscle tissue characteristics will change accordingly as a result of either heat or cold exposure, as few effects were noted, and significant effects did not follow any particular trends, thus no conclusions could be drawn. Despite previous research showing the effects of environmental conditions on muscle tissue characteristics of broilers and turkeys, the results found in the current study are conflicting. Pullets in the current study needed to use energy in order to thermoregulate against exposure to hot conditions; however, thermoregulatory mechanisms employed were adequate to cope with the environmental conditions. Therefore, exposure to the various T/RH conditions had a minimal impact on the well-being of pullets in this study. A D of up to eight hours also had little impact on the welfare of pullets in the current study.

4.0 Chapter 4: Overall Discussion

4.1 Introduction

Though transportation is an essential component of the poultry industry, it can result in stress that may lead to mortality (Mitchell and Kettlewell, 1994). Transportation encompasses various steps, including catching, crating and loading, transport, and unloading (NFACC, 2017). Although many stressors can result from processes during transportation such as handling, noise, vibrations, feed withdrawal, and social disruptions, the most significant stressor during transportation may be the microclimate environment (combination of temperature (T) and relative humidity (RH)) (Broom, 1988; Nielsen et al., 2011). Most transportation trucks in Canada rely on passive ventilation to eliminate heat and moisture from within the trailer (Knezacek, 2005). Because passive ventilation relies on the movement of the truck for air circulation, it can be ineffective at dissipating heat and moisture from within the trailer, especially when tarpaulins are used in cold ambient conditions (Knezacek, 2005; Burlinguette et al., 2012). This potentially ineffective circulation can create T and RH gradients that may subject birds to either heat or cold stress during transportation, depending on the crate location within the truck (Mitchell and Kettlewell, 1994; Knezacek, 2005). Exposure to thermal stress can influence the core body temperature (CBT) of birds during transport, which can put them at risk of either hyperthermia or hypothermia (Knezacek, 2005; Lin et al., 2006; Nielsen et al., 2011). Additionally, long transportation durations (D) can exacerbate poor conditions during transportation, which may lead to higher rates of mortality (Mitchell and Kettlewell, 2009).

Evaluating the welfare of birds during transportation can be difficult. Therefore, it is necessary to use various behavioural and physiological parameters to assess the impact of thermal stress during transportation on the bird's welfare. Evaluating changes in the bird's behaviour can be a useful tool to assess their well-being, as these changes can be indicative of how comfortable the birds feel within their environment (Duncan, 1998). As a response to thermal stress, birds may display thermoregulatory and active behaviours in an attempt to maintain a homeostatic CBT (Duncan, 1998); however, this can be energy costly, leading to, along with dehydration, loses in live weight (Mount, 1979; NFACC, 2017). Additionally, performing thermoregulatory behaviours can result in changes in the blood physiology of the birds, potentially leading to the development of either a respiratory alkalosis or acidosis (Mount, 1979; Thomas et al., 2008; Sherwood et al., 2013). Exposure to either hot or cold T can also

cause altered blood flow to the extremities in order to dissipate or conserve heat for the core body, respectively (Midtgard, 1989). During cold exposure, the extremities may be at risk for frostbite due to vasoconstriction in order to conserve heat for the core body (Midtgard, 1989; Wellehan, 2014). Finally, evaluating various muscle tissue characteristics such as muscle pH, water-holding capacity, and colour post-slaughter can be used to help determine whether birds have been exposed to thermal stress ante-mortem (Pearson and Young, 1989).

Pullets are an essential component to subsequent egg production and are transported at approximately 17 weeks of age from a rearing facility to a laying facility (NFACC, 2017). Despite a large body of literature focusing on broiler transportation, little is known regarding pullet transportation. It is important to understand the implications of thermal stress during transportation and increased journey D on pullet well-being. Therefore, the purpose of this study was to determine the effects of different T and RH combinations, as well as various D, on the welfare of white-feathered layer pullets during simulated transportation. Both hot (30°C) and neutral (21°C) T were used, with a high (80%) and low (30%) RH, as well as a cold T (-15°C, uncontrolled humidity), for a four or eight hour exposure D. To evaluate the effects on pullet welfare, changes in CBT, behaviour, foot T, blood physiology, live shrink, and muscle tissue characteristics were measured. The behaviours observed included various thermoregulatory and active behaviours, which were analyzed using five-minute scan sampling for the entire four or eight hour D. Blood physiology parameters included blood gases (partial pressure of CO_2 (pCO_2), total CO_2 (tCO_2), partial pressure of O_2 (pO_2), and soluble O_2 (sO_2)), acid-base balance parameters (bicarbonate, base excess in the extracellular fluid (BEecf), and blood pH), dehydrations markers (blood sodium, hemoglobin, and hematocrit levels), and the heterophil to lymphocyte (H/L) ratio. Finally, changes in muscle tissue characteristics, including muscle pH, drip loss, thaw loss, cook loss, and colour, were used as determinates of thermal stress pre-slaughter.

4.2 Objectives

The primary objective for this research was to examine the responses exhibited by white-feathered layer pullets when exposed to hot and neutral T, with a low and high RH, and a cold T, for either a four or eight hour D. To complete this objective, changes in the pullet CBT,

behaviour, and physiological responses were assessed. In addition, changes in pullet muscle tissue characteristics were evaluated.

4.3 Discussion

Exposing birds to heat stress during transportation can influence their CBT, which may be a welfare concern. In Canada, depending on ambient conditions, birds within the transport trailer can be exposed to hot T year-round. Knezacek (2005) found that during a transport journey lasting 175 minutes with ambient conditions of 22°C and 24%RH, the conditions within the crates ranged from 22 to 27°C and 11 to 45% RH, depending on the location within the trailer. Knezacek (2005) also noted that during a journey lasting 190 minutes with an ambient T of -27°C, the T within the crates ranged from -10°C to 26°C (no data on RH) depending on their location. Hot T within the trailer can put birds at risk of hyperthermia, especially when in combination with a high RH, as thermoregulation (evaporative panting) in these conditions can be ineffective (Mount, 1979). The data in the current study indicate that pullets exposed to the 30°C 80%RH (30/80) treatment had an initial spike in their CBT; however, through the implementation of behavioural and physiological mechanisms, pullets in this treatment were able to stabilize their CBT (data summarized in Table 4.1). Additionally, exposure to hot T for an extended D can impact pullet CBT. In the current study, pullets exposed for the eight hour D had a 0.53ºC increase in ΔCBT, however, previous research has noted that the normal body T of a bird's CBT can fluctuate without consequence by approximately ±1°C (Donkoh, 1989; Sandercock et al., 2001; Knezacek, 2005; Dadgar et al., 2010). Therefore, a D of up to eight hours had only a minor impact on pullet CBT. This may indicate that the pullets were able to cope with exposure to heat stress and maintain a homeostatic CBT through various behavioural and physiological thermoregulatory mechanisms.

Exposure to cold T during transport is also a concern for bird welfare. A study by Knezacek, (2005) found that during a transport journey lasting 190 minutes with an ambient T of -21°C, the average T within the crates ranged from -14°C to 21°C (no data on RH), depending on crate location within the trailer. Exposure to cold T during transport can influence the CBT of the birds and may put them at risk for hypothermia. The data in the current study demonstrates a depression in the pullets CBT early in the exposure to the -15 treatment. However, by the end of the eight hour D, pullets were able to stabilize their CBT through implementing various

Table 4. 1. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the core body temperature (CBT) of white-feathered layer pullets.

Parameter (°C)	T/RH Combinations	D
Pullet CBT	Lowest in -15	No effect
Baseline CBT	30/30 and 30/80 highest compared to -15	No effect
Δ CBT	No effect	Minimal decrease in the 4 hour D, minimal increase in the 8 hour D

Baseline taken prior to treatment exposure
-15 = -15°C, uncontrolled RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

behavioural and physiological mechanisms. During the last hour of exposure, thermoregulatory mechanisms implemented by pullets exposed to the -15 treatment were inadequate at maintaining homeostasis resulting in a lower CBT (Table 4.1). The results presented by Dadgar et al. (2011) and Dadgar et al. (2012a) agree with that of the current study. Both of these studies found that as a result of exposure to T below -14°C (three hour D), broilers (five to six weeks of age) had a lower CBT, compared to those exposed to T above -13°C (same D) (Dadgar et al., 2011; Dadgar et al., 2012a). The results of the current study indicate that thermoregulatory mechanisms implemented by the pullets exposed to cold conditions were inadequate in stabilizing their CBT. However, the CBT of pullets exposed to the -15 treatment was approximately 38°C, which is still well above the lower critical body T for poultry (19-22°C) (Mount, 1979; Nicol and Scott, 1990).

The data in the current study indicates that blood flow to the extremities was impacted by heat exposure (Table 4.2). When exposed to hot T, vasodilation occurred to increase blood flow and dissipate heat through radiation and convection (Millard, 1974; Mount, 1979; Wellehan, 2014). In order to ensure effective heat dissipation through the skin, pullets aimed to maximize their body surface area exposed to the environment through increasing their time spent surveying and time spent active (Table 4.3). To the best of the author's knowledge, there has been little research done examining the impact of heat stress during transport on pullet behaviour in conjunction with extremity T. Henrikson et al. (2018) found no differences when surveying behaviours of either turkey toms or hens exposed to cold (-18ºC) or neutral (20ºC with 30%RH, or 20ºC with 80%RH) conditions for an eight hour D; however, the environmental conditions in the mentioned study did not exceed 20ºC, as opposed to the current study. When dissipation of heat through non-evaporative heat loss becomes ineffective, pullets must rely on other mechanisms to maintain homeostasis.

Exposure to cold T can also impact blood flow to the extremities. As an adaptation to cold exposure, vasoconstriction of the arteries to the extremities will occur to reduce blood flow and conserve heat for core body (Edwards, 1965; Wellehan, 2014). The data in the present study indicates that cold-induced vasoconstriction may be occurring in pullets exposed to the -15 treatment, as depicted by colder foot T (Table 4.2). There has been minimal research conducted examining blood flow to the extremities in birds exposed to cold conditions. A study conducted by Millard (1974) examined the blood flow to the dorsal pedal artery in the foot of the giant

Table 4. 2. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the foot and comb temperatures of white-feathered layer pullets.

Parameter (°C)	T/RH Combinations	D
Initial Foot Temperature	No effect	No effect
Final Foot Temperature	Lowest in -15	No effect
	Higher in 30/80, compared to 21/30 and 21/80	
Δ Foot Temperature	No effect	No effect

-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/80 = 30°C80%RH

Table 4. 3. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the behaviour of white-feathered layer pullets.

Category	Behaviour (% of time)	T/RH Combinations	D
Activity	Motionless	Increased in -15 and 21/30 compared to 30/80	No effect
Activity	Active	Increased in 30/80 compared to -15	Less time spent active in the 8 hour D
Activity	Shuffle	No effect	No effect
Thermoregulatory	Burrow	No effect	No effect
Thermoregulatory	Shiver	No effect	No effect
Thermoregulatory	Ptiloerection	No effect	No effect
Thermoregulatory	Panting	Increased in 30/30 and 30/80	No effect
Thermoregulatory	Survey	Increased in 30/30 and 30/80 compared to -15	No effect
Pecking	Object peck	No effect	No effect
Pecking	Bird peck	Increased in the 30/30 compared to 20/80 and -15	No effect
Other	Preen	Increased in 21/80 and 30/30 compared to -15	No effect
Other	Head shaking	No effect	No effect
Other	Gulp	Increased in 21/30 and 21/80 compared to -15	No effect
Other	Scratch	No effect	No effect
Other	Undefinable	No effect	No effect

-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH
Behaviour was recorde using 5 minute scan sampling for the entire four or eight hour exposure duration

petrel when submerged in ice water. Millard (1974) found that after 40 seconds of being submerged in the ice water, there was a slight initial decrease in the blood flow to the dorsal pedal artery, after which there was a pronounced decrease in blood flow. The giant petrel is an arctic species and may have a greater adaption to cold T compared to layer pullets; therefore, extrapolation from this study may be difficult. In the current study, final foot T of pullets exposed to the -15°C treatment remained above 12°C, and previous research has indicated that in human skin, frostbite can occur at approximately -2°C (Nagpal and Sharma, 2004). Therefore, vasoconstriction of the vessels to the extremities aided pullets in conserving heat for the core body without putting the extremities at risk for frostbite.

Evaporative heat loss is the primary mechanism for poultry to dissipate heat (Mount, 1979). The results in the current study indicates that in order to maintain a homeostatic CBT, pullets spent more time panting to dissipate heat. Hyperventilation can result in higher amounts of CO_2 being eliminated from the body, causing a shift towards a respiratory alkalosis (Mount, 1979; Sherwood et al., 2013). Interesting to note, pullets in the current study were able to cope with the various T/RH combinations without causing a change in the levels of gases in the blood (partial pressure of CO_2 (pCO_2), total CO_2 (tCO_2), partial pressure of O_2 (pO_2), or soluble O_2 (sO_2)), and without influencing the acid-base balance (blood pH, base excess in the extracellular fluid (BEecf), and bicarbonate) of the body (summarized in Table 4.4-4.5). On the other hand, as a result of panting for a longer D, blood pCO_2, tCO_2, and bicarbonate collectively decreased, suggesting a shift towards a respiratory alkalosis; however, these decreases were not enough to have an impact on the blood pH or blood base excess in the extracellular fluid. Although thermoregulatory behaviours were not measured in previous research, the effect of heat stress on blood physiology has been established in broilers. Sandercock et al. (2001) found that broilers (35 and 63 days of age) exposed to heat stress (32°C and 75%RH) in a climatic chamber for two hours, had a lower blood pCO_2, and a higher blood pH after exposure, compared to the control group (21°C and 50%RH, same D). Ait-Boulahsen et al. (1989) feed restricted broilers (five to six weeks of age) for various D (0, 24, or 72 hours), after which they were exposed to a stepwise increase in environmental T (29-37°C over two hours at a rate of 4°C/hr, then 41°C maintained over an additional two hours). In this study, broilers exposed to heat stress and fasted for 0 or 24 hours, had a higher blood pH, and a lower blood pCO_2, compared to those exposed to heat stress and fasted for 72 hours (Ait- Boulahsen et al., 1989). The findings of the current study disagree

Table 4. 4. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the blood gases, and blood bicarbonate of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Initial pCO_2 (mmHg)	No effect	No effect
Final pCO_2 (mmHg)	No effect	No effect
Δ pCO_2 (mmHg)	No effect	Lower in the 8 hour D
Initial tCO_2 (mmol/L)	No effect	No effect
Final tCO_2 (mmol/L)	No effect	No effect
Δ tCO_2 (mmol/L)	No effect	Lower in the 8 hour D
Initial HCO_3^- (mmol/L)	No effect	No effect
Final HCO_3^- (mmol/L)	No effect	No effect
Δ HCO_3^- (mmol/L)	No effect	Lower in the 8 hour D
Initial pO_2 (mmHg)	No effect	No effect
Final pO_2 (mmHg)	No effect	No effect
Δ pO_2 (mmHg)	No effect	No effect
Initial sO_2 (%)	No effect	No effect
Final sO_2 (%)	No effect	No effect
Δ sO_2 (%)	No effect	No effect

pCO_2 = partial pressure of carbon dioxide
tCO_2 = total carbon dioxide
HCO_3^- = bicarbonate
pO_2 = partial pressure of oxygen
sO_2 = soluble oxygen
mmol/L = millimoles per liter
mmHg = millimeters of mercury

Table 4. 5. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on blood physiology parameters of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Initial pH	No effect	No effect
Final pH	No effect	No effect
Δ pH	No effect	No effect
Initial BEecf (mmol/L)	No effect	No effect
Final BEecf (mmol/L)	No effect	No effect
Δ BEecf (mmol/L)	No effect	No effect
Initial Sodium (mmol/L)	No effect	No effect
Final Sodium (mmol/L)	No effect	No effect
Δ Sodium (mmol/L)	No effect	No effect
Initial Glucose (mmol/L)	No effect	No effect
Final Glucose (mmol/L)	Higher in the 30/80 compared to 21/30 and 21/80	No effect
Δ Glucose (mmol/L)	No effect	No effect
Initial Hematocrit (%PCV)	No effect	No effect
Final Hematocrit (%PCV)	No effect	No effect
Δ Hematocrit (%PCV)	No effect	No effect
Initial Hemoglobin (mmol/L)	No effect	No effect
Final Hemoglobin (mmol/L)	No effect	No effect
Δ Hemoglobin (mmol/L)	No effect	No effect

%PCV= percent pack cell volume
mmol/L = millimoles per liter
21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/80 = 30°C80%RH

with that of previous research. The results from previous studies may differ from that of the current study due to differences in species, exposure conditions, D, and feed withdrawal times. To the author's knowledge, there has been minimal research studying the effect of transport D on the blood physiology of poultry.

Thermoregulatory and stress responses as a result of exposure to heat stress can be energy demanding (Mount, 1979). Initially, birds will use the glucose available in their blood for energy for thermoregulation (Mount, 1979; Sherwood et al., 2013). As this glucose is depleted, energy from the body fat and protein stores will be mobilized and deposited into the blood to be further used for thermoregulation (Mount, 1979; Sherwood et al., 2013). In the current study, pullets exposed to the 30/80 treatment were required to use more energy, as observed by a higher live shrink, likely to not only maintain normal metabolic functions but also to support thermoregulation against these conditions (Table 4.5). On the other hand, pullets exposed to both of the neutral treatments may not have needed to thermoregulate; therefore, the normal metabolic activity could have resulted in a decrease in the final concentration of glucose in the blood. The results of the current study agree with that of Vosmerova et al. (2010) who found that the final blood glucose concentrations of broilers (42 days of age) subjected to 25-35ºC (130 minutes) were higher compared to those exposed to 10-20°C for the same D. The increased mobilization of glucose in response to heat exposure in the current study may indicate that pullets were stressed, and that thermoregulation is energy costly. Therefore, like broilers, increased blood glucose levels can be an indication of poor environmental conditions during transportation of pullets.

In poultry, changes in the heterophil to lymphocyte (H/L) ratio have been established as a measure of chronic stress (Mount, 1979; Sherwood et al., 2013). As a result of an increase in the activity of the hypothalamic-pituitary-adrenal axis, and increased levels of corticosterone, the number of heterophils and/or lymphocytes in the body will either increase and/or decrease, ultimately increasing the ratio (Sherwood et al., 2013; Scanes, 2016). It is interesting to note that in the current study that neither exposure to the various T/RH conditions nor exposure D had an impact on the pullet H/L ratio (Table 4.6). The results of the current study contradict that of previous research. Altan et al. (2010) observed a higher H/L ratio (0.42 vs 0.24) in broilers (35-36 days of age) subjected to 38°C for three hours, compared to those not exposed to heat stress.

Table 4. 6. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the heterophil to lymphocyte (H/L) ratio of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Initial H/L Ratio	No effect	No effect
Final H/L Ratio	No effect	No effect
Δ H/L Ratio	No effect	No effect

The reason for there being no effect of the exposure conditions or D on pullets in the current study is not known. It is hypothesized that these transportation conditions were not enough to elicit a chronic stress response, or that the exposure D was not long enough to observe an effect on the H/L ratio of the well-feathered young egg production pullets.

Along with dehydration, the energy demand of thermoregulation during transportation can cause birds to lose weight (Mount, 1979). Though live shrink is not a direct measure of the bird's welfare, it can indicate the extent of dehydration and energy cost as a result of transportation (NFACC, 2018). In the current study, neither the T/RH combinations nor exposure D had an impact on markers of dehydration, including the levels of hemoglobin, hematocrit, and blood levels of sodium (Table 4.5). Pullets had access to water up until the pre-treatment procedures, this access to water may have been adequate to avoid dehydration during transportation. Thus, it may be suggested that the increased loss in live weight in the pullets exposed to the high T treatments was a result of the gut not only emptying but also the increased energy cost of thermoregulation in these conditions (Table 4.7). Previous research has also shown that as a result of exposure to heat stress, live shrink increases. Petracci et al. (2001) found that broilers (feed restricted 12 hours prior) subjected to 34°C for 12 hours had a higher live shrink compared to those subjected to either 29.5°C or 25°C for the same D. Holm and Fletcher (1997) also observed a greater live shrink in broilers (feed restricted 12 hours prior) exposed to 29°C for 12 hours, compared to those exposed to either 18°C or 7°C for the same D. Therefore, it may be concluded that thermoregulation to counteract heat stress may be energy demanding, causing birds to lose live weight, which may negatively impact their well-being.

Long transport D can further exacerbate the energy costs required to maintain homeostasis in poor environmental conditions. Pullets exposed for the eight hour D were required to thermoregulate for a longer period of time, therefore the energy cost may be greater compared to those exposed for the four hour D. Additionally, a longer period of time without feed may have contributed to the higher live shrink in the pullets in the eight hour D compared to the four hour D (Table 4.7). The results from previous research agree with that of the current study. Bianchi et al. (2005) exposed broilers (38-55 days of age) to various transportation D (<3.5 hours, 3.5-5 hours, or >5 hours), and evaluated the resulting live shrink. The authors found that as a result of transportation for over five hours, the broilers had a greater loss in live weight

Table 4. 7. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6) and different time durations (D) (n=15), on the live shrink of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Initial Body Weight (kg)	No effect	No effect
Final Body Weight (kg)	No effect	No effect
Live Shrink (kg)	Higher in 30/30 and 30/80 compared to 21/80	Higher in 8 hour D
Live Shrink (%)	No effect	Higher in 8 hour D

21/80 = 21°C80%RH, 30/30 = 30°C30%RH, 30/80 = 30°C80%RH

compared to those transported for either of the other D (Bianchi et al., 2005); however, the environmental conditions during transportation were not mentioned. Therefore, research indicates that an increased transportation D can be a welfare concern, as the energy required to maintain homeostasis is higher.

Changes in the muscle tissue characteristics post-slaughter can be compared in order to determine whether birds have been exposed to heat or cold stress ante-mortem (Dadgar et al., 2010; Dadgar et al., 2011; Dadgar et al., 2012a). Changes in muscle tissue characteristics post-slaughter are largely dependent on changes in muscle pH (Pearson and Young, 1989). Previous research has consistently shown that as a result of exposure to either hot or cold T pre-slaughter, the muscle pH post-slaughter will change. With regards to heat stress, Holm and Fletcher (1997) observed a lower breast muscle pH in broilers exposed to 29°C for 12 hours, compared to those exposed to either 18°C or 7°C for the same D. On the other hand, Dadgar et al. (2012a) and Dadgar et al. (2012b) found a higher pH in the breast muscle of broilers (five to six weeks of age) exposed to cold stress pre-slaughter. Dadgar et al. (2012a) subjected broilers to -14ºC for three hours and observed a higher ultimate breast muscle pH, compared to those exposed to T above -10ºC. Dadgar et al. (2012b) also found that compared to those exposed to T above -5ºC, broilers subjected to T below -10ºC had a higher ultimate pH. The results from the current study differ from that of previous research. The data in the current study demonstrates that exposure to the various T/RH combinations was not enough to influence the pH of either the pullet breast or thigh muscle, nor was exposure D enough to have an impact on breast or thigh muscle pH (Table 4.8-4.9). Through the implementation of behavioural and physiological changes, pullets were able to cope with the exposure conditions, thus preventing changes in the pH of the muscle.

Measuring the water-holding capacity of a muscle post slaughter can also be used to evaluate whether birds have been exposed to thermal stress pre-slaughter (Dadgar et al., 2010). Changes in the water-holding capacity are primarily dependent on variations in the pH of the muscle post-mortem (Pearson and Young, 1989). A lower muscle pH resulting from heat exposure pre-slaughter can cause a lower water-holding capacity by decreasing the space between the protein filaments in the muscle (Honikel, 2004). Conversely, a higher muscle pH resulting from cold exposure pre-slaughter can cause a higher water-holding capacity by increasing the space between the protein filaments (Lyon and Buhr, 1999). In the present study,

Table 4. 8. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6) and different time durations (D) (n=15), on the muscle tissue characteristics of the breast muscles of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Left Breast		
Initial Breast Weight (g)	No effect	No effect
Weight Post Thaw (g)	No effect	No effect
Final Breast Weight (g)	No effect	No effect
Thaw loss (g)	No effect	Higher in the 8 hour D
Thaw Loss (%)	No effect	Higher in the 8 hour D
Cook Loss (g)	No effect	No effect
Cook Loss (%)	No effect	No effect
Right Breast		
Initial Breast Weight (g)	Higher in 21/30 and 21/80 compared to 30/30	No effect
Final Breast Weight (g)	Higher in 21/30 and 21/80 compared to 30/30	No effect
Drip Loss (g)	No effect	No effect
Drip Loss (%)	No effect	No effect
Initial pH	No effect	No effect
Final pH	No effect	No effect
Δ pH	No effect	No effect
L*[1]	No effect	No effect
a*[1]	Higher in 30/30 compared to 21/30	No effect
b*[1]	No effect	Lower in the 8 hour D

[1] Muscle tissue colour, n=4 for T?RH, n=10 for D

L* = Lightness

a* = Redness

b* = Yellowness

21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH

Table 4. 9. Summary of the effects of various temperature (T) and relative humidity (RH) combinations (n=6), and different time durations (D) (n=15), on the muscle tissue characteristics of the right thigh muscle of white-feathered layer pullets.

Parameter	T/RH Combinations	D
Right Thigh		
Initial Thigh Weight (g)	No effect	No effect
Final Thigh Weight (g)	No effect	No effect
Drip Loss (g)	No effect	No effect
Drip Loss (%)	No effect	No effect
Initial pH	No effect	No effect
Final pH	No effect	No effect
Δ pH	No effect	No effect
L*[1]	No effect	No effect
a*[1]	Higher in 30/30 compared to 21/30	No effect
b*[1]	Lower in -15 compared to 21/80	No effect

[1] Muscle tissue colour, n=4 for T?RH, n=10 for D

L* = Lightness

a* = Redness

b* = Yellowness

-15 = -15°C, uncontrolled RH, 21/30 = 21°C30%RH, 21/80 = 21°C80%RH, 30/30 = 30°C30%RH

there was no impact of exposure T/RH conditions on breast or thigh muscle drip, thaw, or cook loss, which is to be expected as there was no effect of T/RH conditions on muscle pH (data summarized in Table 4.8 and Table 4.9). The results of the current study are not consistent with that of previous research. A study by Dadgar et al. (2010) found that broilers (five to six weeks of age) exposed to 25ºC for three hours had a higher thaw loss compared to those exposed to T below 15ºC but observed no effect of exposure T on muscle drip loss. Sandercock et al. (2001) exposed broilers (35 days of age) to heat stress (32ºC, 75%RH) for two hours, and noted a higher drip loss in the breast muscle of the birds exposed to heat stress, compared to those exposed to neutral conditions (21ºC, 50%RH). Finally, Dadgar et al. (2012b) observed a higher thaw and cook loss in broilers subjected to 22ºC for three hours, compared to those exposed to T below -9ºC for the same D. In the aforementioned studies, there was an effect of the exposure conditions on the muscle pH which could be correlated with the changes in the water-holding capacity of the muscle (Sandercock et al., 2001; Dadgar et al., 2010; Dadgar et al., 2012b). Contrasting results regarding muscle water-holding capacity between previous research and the current study could be due to differences in bird species, metabolic rates, or feathering. Though there was no effect of the T/RH combinations in the current study on muscle water-holding capacity, there was an impact of exposure D (Table 4.8). However, in the current study, increased transportation D did not have any impact on muscle pH. Therefore, the reason behind this impact of D on muscle water-holding capacity is not well understood, and there has been very little research examining the effect of transportation D on muscle tissue characteristics.

Finally, changes in muscle tissue colour can also be used as an indicator of exposure to thermal stress pre-slaughter. Similar to water-holding capacity, changes in muscle colour are primarily dependent on changes in the muscle pH post-mortem (Pearson and Young, 1989; Pearson, 1994; Dadgar et al., 2010). As the pH of the muscle decreases, it becomes paler, and as the pH of the muscle increases, it becomes darker (Pearson, 1994; Bianchi et al., 2006). Muscle tissue colour can be categorized based on the lightness (L*), redness (a*), and yellowness (b*) values; however, according to previous research, the L* value is the primary determinant of whether muscle is categorized as pale or dark (Dadgar et al., 2011; Dadgar et al., 2012a; Dadgar et al., 2012b). An L* value greater than 53 can be indicative of pale muscle tissue, while an L* value of less than 46 can indicate dark muscle tissue (Dadgar et al., 2011; Dadgar et al., 2012a; Dadgar et al., 2012b). A study by Holm and Fletcher (1997), found that the breast muscle of

broilers exposed to 29°C for 12 hours had a higher b* value, but there was no effect on the a* or L* values. Furthermore, Dadgar et al. (2012a) subjected broilers to various T (-14°C, -9°C, and 21°C) for three hours, and observed that the breast muscles L* and b* values were lower in the -14°C treatment, and the breast muscle a* was highest in the -14°C treatment. In the current study, as a result of the T/RH conditions there were significant differences in the breast muscle a*, and in the thigh muscle a* and b*, however, these differences did not follow a particular trend based on the treatment conditions (Table 4.8 and Table 4.9). Increased exposure D also had an impact on breast muscle b*; however, there has been minimal research done examining the effects of exposure D on muscle tissue colour, therefore the reason behind this difference is not well understood.

5.0 Overall Conclusions

Evidence presented in the current study supports the conclusion that thermoregulatory mechanisms implemented by pullets exposed to heat stress (30°C 30%RH, and 30°C 80%RH) during simulated transportation were adequate in maintaining physiological homeostasis. Evaporative (panting), and non-evaporative (increased activity, surveying, and vasodilation of the blood vessels to the extremities) mechanisms employed by pullets exposed to hot T in the current study were effective at dissipating heat and stabilizing the CBT. However, these pullets were required to use more energy to implement these thermoregulatory mechanisms, as indicated by a greater loss in live weight and a greater final concentration of blood glucose. Additionally, an increased time spent panting resulted in minor changes in the levels of blood gases, but these changes were not enough to impact the blood pH. Finally, exposure to heat stress in the current study caused minor, but non-specific, impacts on pullet muscle tissue characteristics. Therefore, the data presented in this study suggests that pullets were able to adapt to heat stress during simulated transportation with only minor physiological impacts.

With regards to pullets subjected to cold stress (-15°C) during the current study, thermoregulatory mechanisms implemented were inadequate at maintaining homeostasis; however, effects from cold stress, though significant, were minor. In order to conserve energy and heat, pullets exposed to the cold conditions spent more time motionless, and physiological mechanisms, including vasoconstriction of blood vessels to the extremities were implemented. Pullets in this study employed mechanisms aimed to conserve heat, however, a decrease in both extremity T and CBT were noted. Although significant, decreases in the pullet CBT were small, and decreases in extremity T were not concerning. Finally, exposure to cold stress did not affect pullet blood physiology or live shrink and had minimal and non-specific effects on pullet muscle tissue characteristics. Therefore, exposure to cold T only had minor impacts on pullet behaviour, physiology, and muscle tissue characteristics, suggesting that pullets were able to adapt to these conditions with minimal impacts on their well-being.

Thermal stress during transport can be a welfare concern for poultry. Exposure to either heat or cold stress in the current study required pullets to implement behavioural changes and physiological mechanisms in order to either dissipate or conserve heat, respectively. Implementation of these mechanisms caused minimal effects on pullet physiology, and the

resulting changes in muscle tissue characteristics were not well correlated to the exposure conditions created in this study. Finally, a transportation D of up to eight hours resulted in minor impacts on pullet behaviour, physiology, and muscle tissue characteristics. Therefore, the data presented in this study supports the conclusion that between the temperatures and relative humidity ranges studied, pullets were able to adapt to the environmental conditions with minimal impacts on their well-being.

In conclusion, the data outlined in this thesis is important as it provides science-based evidence and an evaluation of the impact of various T/RH combinations, and the impact of increased exposure D, on pullet welfare during simulated transportation. The data in this thesis depicts that exposure to heat or cold stress during transport does influence pullet behaviour and physiology; however, through implementation of thermoregulatory mechanisms, pullets were able to cope with the environmental conditions. Lastly, in this study, a D of up to eight hours had minimal impacts on the pullet's well-being.

5.1 Future Research

There is still limited research on various areas regarding pullet transportation. The current study was a first step in understanding the impacts of the microclimate present during simulated transportation on the welfare of white-feathered layer pullets. However, further research is still required to get a comprehensive understanding of how all aspects of transportation can impact the well-being of pullets. Future research further investigating pullet transportation could focus on variable such as: crate stocking densities, airspeed within the trailer, the impact of stacking crates, the effect of various other stressors during transportation and the combination thereof, and differences in bird strains. Other stressors during transportation that were not included in the present study could include the impact of catching and handling, and vibrations and noise within the trailer. There has also been minimal research examining how exposure to stressors during transportation could impact subsequent bird performance and production.

Within the temperature and relative humidity ranges in the current study, pullets were able to cope with the conditions through the implementation of various thermoregulatory mechanisms. However, further research is needed to gain a full understanding of the development of temperature and relative humidity gradients within the trailer during the real-time transportation of pullets. Monitoring the environmental conditions within the transportation

trailer could aid in establishing a more accurate reflection of the temperature and relative humidity gradients that are present during pullet transportation. Lastly, monitoring the microclimate conditions present within the trailer during transportation could be useful in ensuring temperature and relative humidity conditions are kept within an acceptable range, as not to impact the welfare of the birds during transport negatively.

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7.0 Appendices

Table A. 1. Average crate and chamber conditions achieved for the study (temperature (T) and relative humidity (RH)).

	-15°C		21°C30%RH		21°C80%RH		30°C30%RH		30°C80%RH	
Crate	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)	T (°C)	RH (%)
4 h	-13.13	75.06	24.17	41.55	23.37	73.42	31.77	29.10	32.58	74.53
8 h	-10.90	76.17	23.14	42.51	23.29	73.88	32.11	27.06	32.53	75.28
Chamber	-14.72	69.72	22.05	38.43	21.88	81.39	31.25	29.36	31.49	77.95

Table A. 2. Average transponder readings for the cold treatment and the high temperature, high humidity treatment for each replication.

	-15°C		30°C80%RH	
Replicate	4 Hour	8 Hour	4 Hour	8 Hour
1 (ºC)	40.34	40.16	41.55	39.34
2 (ºC)	40.20	39.72	41.47	39.12
3 (ºC)	40.47	40.57	41.68	41.35