Effects of Simulated Cold and Warm Transport on Turkeys

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by

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ABSTRACT

The effects of cold and warm exposure during simulated transport on 12-week-old turkey hens and 16-week-old toms were assessed in two experiments: a cold-transport analysis with three treatments, -18°C and two 20°C conditions with 30% or 80% relative humidity (RH); and a 2x2 factorial warm-transport analysis comparing the two 20°C treatments with two 28°C treatments, and 30% or 80% RH. Turkeys were crated at 83 kg/m^2 and exposed to conditions for 8 hours before processing. Three replications (8 birds) were performed per treatment for each gender, and between-sex comparisons were made within treatments. Significance was declared at $p \leq 0.05$. Core body temperature (CBT), live shrink (LS), and delta blood glucose (BG) were assessed; meat quality measures included thigh and breast pH and L*, a*, and b* colour values. Behaviour was measured using instantaneous scan sampling during the last 4h of treatment. LS in hens exposed to -18°C (2.9%) was greater than those at 20°C (1.5%). Thigh pH was higher after -18°C exposure (hens: 6.39; toms: 6.08) than after 20°C. In the cold-exposed hens, breast L* values were lower, while thigh a* and breast b* values were higher than in both 20°C treatments. Huddling, shivering, preening, and feather ptiloerection occurred more in cold-exposed turkeys. Between-sex comparison revealed lower LS and a larger decrease in BG in cold-exposed toms; meat characteristics also differed. After warm (28°C) exposure, both hen and tom LS increased, and tom CBT rose approximately 1.0°C. Ultimate breast pH was unexpectedly higher in warm-exposed toms (5.71 at 30%, 5.67 at 80% RH) than those exposed to 20°C (5.71 and 5.69), but lower with increased RH. In hens, initial breast pH increased with warmer temperature, while thigh a* decreased. Several differences in breast pH and a* were noted between sexes within a treatment, and hens had larger BG decreases than toms in both 20°C conditions. In the 28°C 80% RH treatment, LS was higher in hens (3.1%) than in toms (2.44%). Frequency of activity, panting, head-resting, and optional behaviours differed between warm treatments and sexes. Transport conditions (temperature and humidity levels) investigated in this study significantly impacted turkey physiology, meat quality, and behaviour.

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LIST OF ABBREVIATIONS

ACTH	adrenocorticotropic
CBT	core body temperature
CORT	corticosterone
DFD	dark, firm and dry
DOA	dead-on-arrival
EDTA	Ethylenediamine tetraacetic acid
GP	glycolytic potential
h	hour
HLR	heterophil-lymphocyte ratio
HSP	heat shock protein
LS	live shrink
n	number of replications
NEFA	non-esterified fatty acids
PSE	pale-soft-exudative
RH	relative humidity
wk	week

1.0 INTRODUCTION

Transport of livestock is typically necessary at least once in the production cycle. The conditions experienced by food animals during transport are quite variable and can affect some species more dramatically than others. Poultry are considered susceptible to thermal stress, a problem which is frequently encountered during transport. Heat stress in broilers has been much more thoroughly characterized than cold stress, but neither form of thermal challenge has been sufficiently researched in the domestic turkey, *Meleagris gallopavo.* In order to implement effective solutions to welfare and productivity problems which arise during transport, it is necessary to characterize the environment and the stressors encountered by different livestock species (Mitchell and Kettlewell, 1998).

In Canada, approximately 22 million turkeys are transported per year, while around 10 times that are transported in the USA (National Agricultural Statistics Service, 2014, Agriculture and Agri-Food Canada, 2016). The duration of transport varies based on distance from farm to processor, but Section 5.5 of the Canadian Codes of Practice for poultry production simply recommends duration be less than 36 hours (National Farm Animal Care Council, 2016). External temperatures in Canada can range from -40°C to 40°C from winter to summer, but in Ontario and Quebec where the majority of turkeys are produced, the annual low is around -25°C, while the summer high is 17°C and 26°C, respectively (Environment Canada, 2016). No specific transport temperature thresholds are mentioned in the Codes of Practice (sections 5.3 and 5.4), but it is advised that animals be protected from severe weather conditions, and precautions are recommended for both cold and hot or humid weather, including avoiding transport when necessary (National Farm Animal Care Council, 2016). Trailer conditions are largely dependent on these external conditions as outside air flows in, and they are also affected by airflow rate and the heat and moisture production of the birds (Kettlewell et al., 2000; Knezacek et al., 2010). There is a significant link between DOAs and the trailer microclimate, with increases in both warm and cold months, as well as a link between mortality and transport distance or duration (Warriss et al., 1992; Hunter et al., 1997; Nijdam et al., 2004; Vecerek et al., 2006; Voslarova et al., 2007).

Thermal stress may be experienced by birds being transported at cold temperatures, where

both extreme heat and cold can occur within the same load. Attempts to protect birds from cold external temperatures may lead to reduced ventilation within the trailer and the development of a central core of high temperatures, while the periphery may only be a few degrees warmer than the external temperature (Knezacek et al., 2010; Burlinguette et al., 2012). Management strategies, like adjusting loading density according to temperature, could improve the birds' ability to thermoregulate, and decrease DOAs (Poultry Industry Council, 2010), but this may increase transportation costs, and it is time-consuming to implement in the field. In addition to DOA losses and the welfare implications of thermal stress, further economic losses result from condemnations at the slaughter plant. Heat stress, which can occur during both warm and cold transport, is typically associated with pale, soft, exudative meat (PSE), while cold stress can result in dark, firm and dry meat (DFD) (Mallia et al., 2000; Owens et al., 2000). Though DFD traits may not directly compromise meat quality, both DFD and PSE defects affect consumer acceptance, and thus the excessively thermally stressed bird may be a loss even if it survives its journey.

The ability of poultry to cope with the stress of transport has been shown to be affected by a number of factors including environmental, management, and bird-related aspects. This includes health status prior to transport, feed and water withdrawal times, handling practices, trailer design, loading density, external environmental conditions, transport distance and duration, lairage conditions, and importantly, the microclimate within the trailer (Schwartzkopf-Genswein et al., 2012). These variables not only affect bird welfare and physiology, but also live weight, meat quality, numbers of birds dead on arrival (DOA), and condemnation rates (Schwartzkopf-Genswein et al., 2012), indicating that there are both ethical and economic motivation to regulate and improve this facet of poultry production. Relevant research evaluating these components of cold transport in broilers has begun to emerge, but the response of turkeys to both cold and warm transport conditions has not been equally explored. Guidelines for researchers examining welfare during the transport process, set out by Mitchell and Kettlewell in 1998, include characterizing major stressors and their most responsive physiological indicators, using stress profiles to determine acceptable limits for different stressors, examining interactions amongst stressors, testing lab models under field conditions, and finally, designing strategies to alleviate or prevent the stress experienced through improved transport practices, regulations, and vehicle design.

1.1 Justification for Research and Measures

As turkeys are transported year-round in North America, they stand to be exposed to a wide range of temperatures, including hot summer temperatures and extreme winter cold. The potential impact of temperature extremes on turkeys during transport has been suggested based on data for broilers, but even this research is mainly focused on heat stress. Characterizing the effect that cold and warm transport has on indicators of stress in turkeys is an important step in setting standards to improve transport conditions. While the effects of thermal stress may not always be undesirable (such as changes in meat quality parameters after cold transport), characterizing these changes is a critical part of harnessing the benefits and reducing the harms. In order to improve any production system, the process must be observed and assessed at all stages, so important factors can be isolated. At this point, insufficient research exists to accurately evaluate problems which arise during transport in turkeys, severely limiting the actions which can be taken to correct any issues. Determining what effects transport at both cold and warm temperatures have on indicators of turkey behaviour and physiology will serve as one of the first steps to improving both welfare and productivity during this necessary event.

Evaluating the thermoregulatory abilities of turkeys and determining the points at which welfare is negatively affected or coping ability is exceeded allows for the development of more effective techniques for reducing thermal stress. Measuring behavioural, metabolic and physiological changes can be useful for setting the boundaries of productive and humane transport. The biochemical changes which occur during the transport process can have wide ranging effects on factors such as live shrink, condemnations, and meat quality – measures which have value to producers, transporters, and processing plants. Improving the condition of birds arriving at the processing plant not only improves productivity and quality, but also reduces mortality and waste.

In order to assess the impact of different transport conditions on male and female turkeys, several physiological, meat quality, and behavioural measures were analyzed during a simulated transport event. The temperature levels used were selected based on both expected real-world conditions and past research, while high and low humidity levels of 80% RH and 30% RH were used to help clarify the role of humidity in thermal stress. The -18°C treatment (humidity

uncontrolled) reflected common winter transport temperatures in Canada, while the 20°C treatments were to serve as a control, as this temperature is close to the recommended barn temperature for turkeys of near-market age, 18°C (Hybrid Turkeys, 2016). The warm temperature selected to induce heat-stress was originally 35°C, based on prior broiler research, but was scaled back to 28°C due to unexpected tom mortality (Gabriel et al., 1996; Altan et al., 2003). Physiological measures used in this study included live shrink (LS), heterophil-lymphocyte ratio (HLR), and changes in core body temperature (CBT) and blood glucose before and after the treatment. Meat quality measurements included initial and ultimate breast pH, ultimate thigh pH, and the breast and thigh colour values L* a* and b*. Behavioural measures included time budgets of position, activity, thermoregulatory, and other 'optional' or social behaviours, such as preening and pecking. The varied physiological, behavioural, and meat quality measures used in this research allow for a multifactorial view of the impact of thermal challenge on elements of welfare and economic relevance.

Though thermal stressors during transport have the potential to severely detriment welfare, there are several other transport-related stressors to which turkeys in this study were not exposed. Air speed in the environmental chambers was near zero (though fresh air was continuously supplied), while in a real transport event birds may be exposed to highly varied air speeds from both the movement of the truck and wind. Transported turkeys may also be exposed to adverse weather conditions, including rain or snow, which can impact thermoregulatory abilities. The motion, vibration, and noise of a transport truck were also absent, though the environmental chambers were not silent. Despite these differences, the responses of turkeys to various climactic conditions while crated at industry-standard densities was expected to provide information relevant to the transport process.

1.1.1 Physiological Measures

The physiological measures selected for this study allow for some inferences to be made about the metabolic state and stress response of turkeys exposed to the different transport conditions. Live shrink (a percent value of the live weight loss experienced during transport) not only represents a potential economic loss, but can also give some indication of the catabolic changes resulting from feed deprivation, dehydration, and thermoregulation. Some previous

research indicates that both hot (34°C) and cold (-4°C to -18°C) conditions increase live shrink in broilers beyond that experienced at temperatures closer to thermoneutral, in these cases, 25°C and 20°C (Petracci et al., 2001; Dadgar et al., 2011). This may be due to energy spent on thermoregulatory behaviours (i.e. panting) and body heat production, respectively (Schwartzkopf-Genswein et al., 2012).

Core body temperature, which is homeostatically regulated in avian species, has also been demonstrated to be affected by cold and warm temperature exposure. Among turkeys, normal CBT is approximately 40.3 to 40.8°C (Mills et al., 1999; Yahav et al., 2008). The CBT of broilers appears negatively affected by cold temperatures of -10°C and -15°C, but unaffected by exposure temperatures of around -5°C, though this threshold is warmer if birds are wet (Hunter et al., 1999; Strawford et al., 2011). Broiler data indicate that when external temperatures are above 23°C, heat stress can occur, with accompanying rises in CBT and a 6.6-fold increase in mortality rates (Warriss et al., 2005). When core body temperature deviates from baseline levels, it indicates that thermoregulation mechanisms are insufficient to cope with exposure temperatures. While this may represent a welfare issue, it is also related to the additional energy demand imposed on poultry transported at adverse temperatures – declining CBT is correlated with decreases in blood glucose and muscle glycogen reserve, both indicators of energy availability (Dadgar et al., 2011; Dadgar et al., 2012). In contrast, heat-stressed broilers tended to experience an increase in blood glucose, potentially influenced by a stress-related increase in glucocorticoids, and total plasma protein and triglycerides were decreased (Borges et al., 2004; Vosmerova et al., 2010). Glucose changes during cold and warm exposure are expected to contribute to the understanding of the metabolic effects of different transport conditions.

The heterophil-lymphocyte ratio has often been used as an indicator of chronic environmental, social, and thermal stressors (Gross and Siegel, 1983; Maxwell, 1993; Zhang et al., 2009). In broiler research, short bouts (3h) of heat stress and rising microclimate temperatures during transport have reliably resulted in an increased HLR, via the effects of adrenocorticotropic hormone (Maxwell, 1993; Mitchell and Kettlewell, 1998; Altan et al., 2003). The effects of cold transport on HLR have not been directly measured, but exposure to cool housing temperatures of 6°C for one day did result in higher HLR ratios in broilers (Gross, 1988). Taken together, these physiological indicators are expected to give an idea of the

magnitude and type of effect on metabolic status and biological stress of turkeys transported at warm and cold temperatures.

1.1.2 Meat Quality Indicators

Meat quality indicators provide additional information on the physiological impacts of thermal stress, in addition to their primary use in determining the impact on palatability, cooking, and processing characteristics. Indicators used in this study included: initial and ultimate breast pH, ultimate thigh pH, and the breast and thigh colour values L* (lightness) a* (redness) and b* (yellowness). In some previous broiler and turkey research evaluating heat stress, muscle pH has declined alongside detrimental quality changes such as decreased water holding capacity, increased toughness, and often lighter meat colour with a higher L* value, though this is not always observed (McKee and Sams, 1997; Petracci et al. 2001; Bianchi et al. 2005; Bianchi et al. 2006; Petracci et al., 2006; Dadgar et al., 2010). The observed changes in meat characteristics arise when pH drops due to the degradation of glycogen into lactic acid, and colour lightens as muscle proteins are denatured. This quality issue has been equated with a similar condition in pork, known as PSE – for pale, soft, and exudative meat (Sams, 1999). Cold exposure, in contrast, has been shown to increase muscle pH and water-holding properties in broilers, with darker, redder meat and a higher a* value, though the effects on toughness are not conclusive (Lee et al., 1976; Boulianne and King, 1998; Dadgar et al., 2010; Dadgar et al., 2011). Marinade pick-up, drip-loss, and cook-loss are improved in darker coloured broiler breast meat, but odor and shelf life may be negatively affected – the direct relationship of these measures with cold exposure is less clear (Allen, Russel, and Fletcher, 1997; Allen et al., 1998). While differences between turkey and broiler meat colour characteristics limit the direct usefulness of broiler research (Werner et al., 2009), meat quality indicators are expected to change similarly between species in response to thermal stress. Because changes in pH and meat colour often occur alongside changes to more direct measures of meat quality, these indicators were selected to assess the impact of thermal stress in the present study.

1.1.3 Behavioural Assessment

Behavioural measures, and in particular, time budgets, are widely used in welfare research to assess time spent performing normal, abnormal, and coping behaviours in new or adverse events and conditions (Sherwin and Kelland, 1998; Alvino et al., 2009). If behaviours used to cope with thermal challenge increase at the expense of other normal and 'optional' behaviours, and occur alongside other biological changes indicating insufficient coping, then they may be used to assess the impact of adverse thermal conditions. Specific behavioural markers which occur during cold or warm exposure may serve as non-invasive methods of gauging the level of thermal stress experienced by poultry during transport and in the barn, while a decrease in 'optional' behaviours such as preening and pecking may indicate a negative impact on welfare or affective state (Weeks et al., 2000; Wathes et al., 2002; Pereira et al., 2007).

Of the behaviours recorded, several were expected to be directly related to thermoregulation, as supported by previous broiler and turkey research. Huddling behaviour and ptiloerection of feathers, as well as the obvious shivering, have been demonstrated responses to cold exposure in broilers (Whittow, 1976; Strawford et al., 2011). During heat stress, panting is an important mechanism for thermoregulation, though its effectiveness may be reduced in high humidity conditions. Exposing skin to increase heat loss by lifting or drooping wings to expose the bare skin of the abdomen may be seen in heat-stressed broilers and exposure of the bare head and neck among turkeys further aids thermoregulation in this manner (Buchholz et al., 1996; Warriss et al., 2005).

1.2 Objectives

Determining which indicators are responsive to thermal stress may help to determine the threshold of acceptable transport conditions and allow one to determine which management and equipment changes have the most benefit in reducing thermal stress. The response to thermal stress must be well-characterized before changes that measurably benefit transported birds can be made. Determining the point at which welfare is negatively affected will also allow for better and more accurate regulations governing poultry transport. Improvements to transport conditions have the potential to benefit consumers, producers, and processing plants through improved welfare and consistency, and reduced loss and waste.

The objectives of this research were to:

1. Determine how simulated cold and warm transport affect turkey hen and tom behaviour and selected physiological indicators of stress and welfare, and

2. Determine how simulated cold and warm transport affect selected indicators of turkey hen and tom meat quality.

It was hypothesized that a thermally adverse transport environment would result in a decrease in blood glucose and live weight, an increase in heterophil-lymphocyte ratio, and an inability to maintain baseline core body temperatures. Cold exposure was also expected to result in changes to meat quality after slaughter, including darker colour and increased initial and ultimate pH, while warm exposure would cause lighter colour and decrease initial and ultimate pH. Behavioural changes, including time spent engaged in active, inactive, thermoregulatory, and optional behaviours, were also expected to occur under adverse thermal conditions. Cold exposure was expected to result in more shivering, ptiloerection, and huddling behaviours, with a possible increase in movement or activity levels, while warm exposure was expected to produce more panting and a decrease in activity. Both exposure conditions were expected to cause a decrease in frequency of optional or comfort behaviours, such as preening and pecking. Hen and tom data, which were expected to differ based not only on sex but also their differing age and size, were nonetheless compared. Despite these confounding effects, the comparison was one of birds both at their respective market ages, and thus was expected to provide information on their different responses to some typical experiences during transport.

2.0 LITERATURE REVIEW

2.1 Trailer Microclimate

The microclimate within the trailer is considered one of the most important factors affecting productivity and welfare during transport. Thermal stress contributes to the overall stress experienced during transport, and can prove fatal, accounting for up to 40% of broiler DOAs (Mitchell and Kettlewell, 1998). Uniform, thermoneutral temperatures allow birds to more easily thermoregulate and preserve health, body condition, and welfare compared to extremely cool or warm temperatures. However, the microclimate within a trailer is often unevenly distributed and poorly controlled, which means at least some portion of birds may be exposed to suboptimal conditions even if other parts of the trailer are an ideal temperature. When humidity is high or birds are wet, higher mortalities result due to decreased efficiency of panting and feather insulation, respectively (Mitchell and Kettlewell 1993; Hunter et al., 1999; Schwartzkopf-Genswein et al., 2012). Both hyperthermia and hypothermia can occur in the non-uniform trailer microclimate (Knezacek et al., 2010), which is largely affected by the external temperatures and the configuration of curtains and vents. Densely packed modules within the trailer can obstruct airflow and mixing, with air speed and unplanned air inlets further affecting the temperature gradient (Knezacek et al., 2010). This variability in temperature throughout the load is much more pronounced during winter months, when external temperatures drop. Then, the development of a hot inner core behind the headboard, with temperatures possibly exceeding 30°C (Knezacek et al. 2010), may occur alongside peripheral near-ambient temperatures of below -20°C (Burlinguette et al., 2012). Uniformity can be improved with active ventilation but it is likely that further changes, both to trailer design and transport practices, are required to stabilize the microclimate in colder geographic regions.

Conditions within the trailer are also affected by non-external factors, such as loading density and bird behaviour. As birds attempt to avoid both warm and cold stressors (MacCaluim et al., 2003), they move within crates and drawers and their uneven distribution can cause further thermal inconsistency (Strawford et al., 2011). The amount and dispersal of heat and moisture produced by the flock must also be taken into account. The heat production rate of modern broilers has increased alongside their rapid growth and metabolic rates, while their ability to tolerate temperature variations has simultaneously decreased (Deeb et al., 2002; Watts et al., 2011). Heat production in broilers is further affected by light intensity (Aerts et al., 2000),

feeding regimen (Koh and Macleod, 1999), physical activity levels (Saiful et al., 2002), and age (Xin et al., 2001). As one would expect, exposure temperature significantly affects heat production and somewhat affects moisture production as birds attempt to thermoregulate accordingly (Watts et al., 2011). Further confounding effects, such as decreased ventilation when the trailer is stopped for loading or lairage (Mitchell and Kettlewell, 1998), the quality and distance of the drive (Voslarova et al., 2007), and variations in the height of the modules within the trailer (Wichman et al., 2012) make determining the ideal transport situation difficult. The determination of an optimum loading density, given variations in bird size, age, sex, and external temperatures is an important goal in improving bird performance and welfare during transport, but this is further complicated by variations in temperatures within the vehicle. Employing active ventilation and understanding the relationship between heat and moisture production, physiology, and behaviour will allow for improved transport conditions, decreasing losses due to death, condemnations, and shrinkage (Watts et al., 2011).

2.2 Physiology and Thermal Stress

The physiology of the heat-stressed broiler has been studied extensively, but information on the effect of cold stress in broilers is only recently being pursued. Research on thermal stress during transportation of turkeys is even less prevalent, and so assumptions have been made by producers and the industry about the similarity of these species' reactions and thresholds for thermal stress. While depending on broiler data when assessing the transport of turkeys lends a starting point for researchers, efforts to elucidate the differences between these poultry species will prove important in developing accurate strategies for reducing stress and meat quality defects.

During transport, the highly varied microclimate results in different states of physiological distress occurring simultaneously. Transport itself, even at thermoneutral temperatures, is a major stressor which causes measurable physiological changes. Separating stress due to transport from stress caused by extreme temperatures will allow for evaluation of how detrimental thermal stress alone is to welfare and meat quality, paving the way for targeted solutions. Without thermal stress, transport still has major effects on various hematological, enzymatic, and hormonal parameters in broilers. The length of transport and feed withdrawal have a negative relationship with blood glucose, as energy stores in the blood, and later, liver and muscle

glycogen, are gradually depleted (Zhang et al., 2009). These changes are mainly mediated by the actions of insulin, but are also affected by the stress hormone corticosterone (CORT). An increase in CORT is an indicator of acute stress, and it is strongly affected by catching and handling prior to transport, before gradually decreasing as birds settle within the trailer (Vosmerova et al., 2010). The release of CORT into the bloodstream via the adrenocorticotropic hormone (ACTH) affects muscle density, and enhances both lipolysis and glycolysis (Zhang et al., 2009). These processes work to free up energy in the stressed and feed-withdrawn broiler, in the form of lipids from body fat and glycogen from the liver and muscles. ACTH also brings about an increase in the heterophil-lymphocyte ratio, a slower-acting and more sensitive measure of stress, which is less affected by handling (Zhang et al., 2009). This ratio is a reliable indicator of stress in birds, and is affected by many stressors, including temperature (Gross and Siegel, 1983; Altan et al., 2003). Meat quality changes are also affected by corticosterone, which is thought to be an important driver in the reduction of type 1 and type 2a muscle fibre area and density (Zhang et al., 2009), in addition to the liberation of muscle glycogen stores. Recovery time reduced the corticosterone-mediated effects on meat quality, but after 3 hours neither corticosterone nor muscle metabolism fully declined to pre-transport levels (Zhang et al., 2009). Plasma levels of non-esterified fatty acids (NEFA) are also affected by transport. A rise in plasma NEFA occurs after available blood glucose has been depleted and lipolysis has begun, and thus is mainly affected by feed withdrawal (Zhang et al., 2009). Other measures of metabolic stress, such as plasma uric acid, lactate, triglyceride, creatine kinase, and plasma protein levels have less clear roles in the process of thermal and/or transport stress, and may be largely affected by other factors. Lactate and creatine kinase can be useful markers of stress due to physical activity and handling, which can also have important welfare and meat quality implications due to the potential for muscle damage associated with their increase (Vosmerova et al., 2010).

Heat Stress. When a bird can no longer control body temperature without altering metabolic rate and behaviour, it has entered into non-thermoneutral conditions. The heat-stressed broiler experiences greater stress than one transported at thermoneutral temperatures, as indicated by an increasing heterophil-lymphocyte ratio with rising microclimate temperature (Mitchell and Kettlewell, 1998). Even short, two-hour exposures to heat will produce increases in the HLR at high temperatures of 39°C (Warriss et al., 2005). Hyperthermia in the form of

elevated CBT is frequently found during summer transport and in the centre of winter-transported loads, as low ventilation levels allow for rising temperatures and humidity, both of which inhibit the thermoregulation abilities of the birds (Schwartzkopf-Genswein et al., 2012). In addition, excessive thermal panting can lead to respiratory hypocapnia as falling plasma CO_2 levels disrupt the acid-base balance with alkalosis (Mitchell and Kettlewell, 1998). The increase in mortality due to heat stress is often associated with this acid-base imbalance, alongside the direct effects of hyperthermia.

The heat shock proteins (HSP), a highly-conserved set of proteins found in many species, are useful indicators of thermal stress. In broilers, and presumably turkeys, some of the HSP have a protective role during episodes of heat stress. They are also associated with a wide variety of other non-environmental stressors. HSP are rapidly synthesized in response to extreme temperatures and may act to prevent myocardial injury and maintain cellular structural integrity (Yu et al., 2008). Variations in HSP70 expression among transport-stressed broilers have been correlated with indicators of meat quality (lightness and pH) as well as stress-associated enzymes such as creatine kinase (Xing et al., 2017).

Cold Stress. Cold-stressed broilers may experience increased stress compared to those transported at thermoneutral temperatures and even warm temperatures, evidenced by increased corticosterone levels (Vosmerova et al., 2010). Additionally, the decrease in plasma protein found in transported birds is more pronounced at cold exposure temperatures of about -5°C, compared to moderate and high exposure temperatures ranging from 10 to 35°C (Vosmerova et al., 2010). This effect may be due to increased metabolic demands liberating energy stores from the muscles and liver. Blood glucose levels seem to decrease on exposure to cold temperatures in broilers (Dadgar et al., 2011), but evidence is conflicting. In one study on the biochemical changes in turkeys after 4 days of mild cold exposure, blood glucose was found to increase compared to control temperatures (Aarif and Mahapatra, 2013). The observed decrease in blood glucose levels, as well as NEFA and triglycerides, may however simply be due to the normal metabolic demands of feed withdrawal during transport rather than thermoregulation. The glycolytic potential (GP) has also been observed to decline significantly in highly cold-stressed broilers (Dadgar et al., 2011). This measure of current and potential lactate production from carbohydrates indicates birds' ability to cope with increased energy expenditure. The steady

decline of the GP as well as CBT, more pronounced in males, implies that birds are not meeting their energy demands (Dadgar et al., 2011).

Cold transport also comes with the increased risk of moderate or severe hypothermia. This risk is exacerbated if birds become wet during transport, whether by road spray, the birds above them, or being loaded wet from the barn (Hunter et al., 1999). Hunter et al. also reported, based on work by Freeman (1971), that flock mortality is increased if CBT drops below 32°C, and CBT of 24°C or below tends to be lethal in individual birds.

Heat shock proteins are also affected by cold stress, with several variants decreasing, while the variants HSP70 and HSP90 increased (Yu et al., 2008; Zhao et al., 2013). HSP90 showed the opposite trend under heat stress, implying that it may have a role in protecting against the negative effects of hypothermia (Yu et al., 2008). Episodes of cold stress in broilers can cause significant damage to the heart, including tissue lesions and ruptured myocardial fibre, as well as the migration of inflammatory cells to the oxidation-damaged areas (Zhao et al., 2013).

Another negative impact of cold stress on the cardiovascular system of fast-growing broilers, which results from increased oxygen demand of metabolically active tissues, is ascites. Hypoxia of these active body tissues can occur, and the heart may become congested as it struggles to meet the growing oxygen demand. As heart rate and cardiac output increase, so does hematocrit (packed cell volume), hemoglobin level, and red blood cell count (Ipek and Sahan, 2006), in order to improve the oxygen-carrying capabilities of the blood. Continued hypoxia due to cold stress will result in pulmonary hypertension and fluid accumulation in the pericardium and abdominal cavity, the classic and condemnable markers of ascites, though predisposition and subclinical ascites before transport are important factors. This condition can result in both welfare and economic losses, making it an important motivator in understanding and limiting cold stress during transport.

CBT. Core body temperature is a simple way of measuring whether or not a thermally stressed bird is able to compensate for extreme conditions. Like all warm-blooded creatures, poultry will attempt to keep CBT static, regardless of the ambient temperature of their surroundings. Among turkeys housed at recommended temperatures, a normal CBT is around 40.3 to 40.8°C (Mills et al., 1999; Yahav et al., 2008). CBT has a positive relationship with exposure temperature, with greater decreases observed at colder temperatures (Strawford et al.,

2011). It has not been scientifically determined exactly what the lowest acceptable exposure temperature is from a welfare standpoint. Individual broilers exposed to -5°C for 3 hours seemed to cope adequately, while broilers at -10°C and -15°C had a CBT which would be considered hypothermic by Richards' characterization in 1977, as reported by Strawford et al. (2011). Though the rate of decline of CBT is reduced the longer the cold conditions persist, CBT does not fully rebound after 2 hours of lairage time (Strawford et al., 2011). Declining CBT in cold-stressed broilers is correlated with blood glucose decreases, and the accompanying depletion of muscle glycogen reserves suggests there is a lack of energy available for thermoregulation (Dadgar et al., 2011; Dadgar et al., 2012). Wet broilers, as mentioned before, are more susceptible to cold stress than dry comparators. Wet broilers were found to experience a drop in CBT at temperatures around +8°C, while dry birds could withstand -4°C without any decrease in CBT (Hunter et al., 1999). Exposure to high temperatures of 32.5 - 35°C can also elicit changes in broiler CBT, as well as skin temperature, which has been correlated with CBT (Yahav et al., 1997; Sandercock et al., 2001; Berri et al., 2005; Lin et al., 2005). This effect is further amplified when both temperature and humidity are high (35°C and above 60% relative humidity), and may be a result of reduced effectiveness of evaporative heat loss (Sandercock et al., 2001; Lin et al., 2005). However, at temperatures of 20°C - 30°C, the effects on broiler body temperature are less pronounced or insignificant, and the impact of humidity is suppressed (Lin et al., 2005; Dadgar et al., 2010).

Live Shrink. Live shrink refers to the weight loss which occurs after birds leave their home barn, prior to slaughter. It is an economic issue, but may also be related to welfare compromise, with broilers losing from 3% to 5% of their body weight in a 12-hour span (Lyon et al., 1991; Northcutt et al., 2003). Unfortunately, literature on expected live shrink in turkeys, and the expected effect of transport temperature on such, is sparse. However, one study by Duke et al. in 1997 suggested similar shrink losses to broilers, approximately 0.2 – 0.4% per hour of feed withdrawal, or between 2 and 5% in a 12-hour span. Studies have found that both hot and cold conditions significantly increase live shrink in broilers (Petracci et al., 2001; Mitchell et al., 2003; Dadgar et al., 2011), but other contrary evidence attributes this to transport alone (Nijdam et al., 2005; Aviagen, 2009). It was suggested by Schwartzkopf-Genswein et al. (2012) that additional weight during cold transport may be lost due to the increased energy demands of

producing sufficient body heat. In contrast, during heat stress, it is panting which may raise energy demands as well as cause dehydration (Whiting et al., 2007; Schwartzkopf-Genswein et al., 2012). Clarifying the effect of thermal stress on live shrink is important for bird welfare but also allows one to determine conditions that promote the lowest shrink loss after transport, benefiting producers and processors.

2.3 Meat quality

The physiological stress of transport not only affects the living broiler or turkey, but may also result in changes to meat quality. The effects transport stress have on glucose, glycogen, and other metabolite levels can combine with the effects of heat and cold stress to produce condemnable meat quality defects. For a variety of environmental stressors, thigh meat seems to be more dramatically affected than breast meat, possibly due to a greater effect of temperature on the more peripheral muscle (Debut et al., 2003; Dadgar et al., 2011). Reported meat quality indicators in transported turkeys vary between studies. Among those exposed to transport at thermoneutral temperatures, initial breast pH can range from 6.07 to 6.19; ultimate breast pH can range from 5.70 to 5.77; and breast colour values, L*, a*, and b*, can vary from 50.96 to 51.97, 13.61 to 14.13, and 3.89 to 7.69, respectively (Werner et al., 2009; Boukhris et al., 2017).

Heat stress seems to result in accelerated rigor mortis, post-mortem glycolysis, and other undesirable metabolic changes which can result in pale, soft, exudative (PSE) meat (Sams, 1999; Zhang et al., 2009). As reviewed by Schwartzkopf-Genswein (2012), most research on heat-stressed birds also shows a decline in pH, water holding capacity, and results in tougher, paler meat, in both broilers and turkeys (Mckee and Sams, 1997; Aksit et al., 2006), though the effect on colour is diminished in turkeys and at more moderate temperatures of 20 to 30°C (Froning et al., 1978; Northcutt, 1994; Dadgar et al., 2011).

In contrast, cold stress in broilers can result in the dry, firm, dark quality defect, as a result of the reduction in ante-mortem and post-mortem muscle glycolysis. This meat defect is characterized by higher pH and darker colour, as indicated by lower L* (lightness) and b* (yellowness). This high-pH meat has a* (redness) values similar to normal or moderate pH meat (Chan et al., 2011). DFD or high-pH meat, though considered a defect, may have improved water

holding properties in turkeys and broilers (Dadgar et al., 2010; Dadgar et al., 2011; Strawford et al., 2011). Additionally, the higher-pH meat observed in cold-stressed poultry has been shown to result in larger yield, reduced drip loss, and improved texture and taste scores for white meat (Fernandez et al., 2002). Some of these properties are beneficial for further processing, but colour variations could cause consumer rejection, and high pH may decrease shelf life (Chan et al., 2011; Schwartzkopf-Genswein et al., 2012). Studies examining the tenderness of cold-stressed broiler and turkey meat have produced conflicting results. While some of the characteristics are associated with improved meat quality, after a certain point they become detrimental to consumer acceptance. For cold stressed broilers, the point at which meat quality was observed to decline most noticeably was at exposure temperatures below -14°C, where DFD incidence reached 60% in immobilized birds (Dadgar et al., 2011). Lairage time after transport can cause a further increase in DFD, as the metabolic deficit continues in the absence of feed (Dadgar et al., 2011). DFD traits are found among turkeys condemned for cyanosis, which follow a seasonal pattern, with greater incidence in colder months (Mallia et al., 2000). Stress, dehydration, food withdrawal, and cold temperatures can contribute to this major cause of condemnations, and the condition can be considered indicative of stress (Mallia et al., 2000). The incidence of DFD traits increases with lower crating densities, supporting a thermal component, and emaciation or longer durations of feed withdrawal can also contribute to glycogen depletion and exhaustion (Warriss et al., 1990).

2.4 Stress, Behaviour and Welfare

Recognizing, quantifying, and determining the relationship of physiological stress with welfare are important steps in assessing where acceptable limits lay for a variety of stressors. The characterization of a stress profile for each stressor is an important and time-consuming endeavor, but it is necessary in order to be confident that birds' performance and well-being are not excessively compromised. The physiological effects of thermal stress can be significant, and some markers in particular are more typically associated with welfare than others. Consideration of the functional significance of biomarkers, and the behavioural responses observed, can be useful in determining which marker is most relevant to a given stressor (Mitchell and Kettlewell, 1998). However, defining and recognizing the degree of suffering can be difficult. Welfare among farm animals is often discussed in the context of the Five Freedoms, briefly: freedom

from hunger and thirst; freedom from discomfort; freedom from pain, injury, or disease; freedom to express normal behaviour; and freedom from fear and distress (Farm Animal Welfare Council, 1992). Animal welfare has also been described by Fraser (2008) as the intersection of three factors: basic health and functioning, including freedom from injury and illness; the affective states of an animal, with consideration for both negative and positive affect; and the opportunity for animals to live relatively natural lives. All of the Five Freedoms and the three themes discussed by Fraser have the potential to be negatively affected by the transport process.

Transported poultry are almost always exposed to a large number of novel stressors, including temperature changes, the motion and noise of the vehicle, fasting and water withdrawal, and social disruption (Mitchell and Kettlewell, 1998). The effects may range from mild discomfort to distress and even death, with up to 40% of DOAs attributed to transport stress (Bayliss and Hinton, 1990). Confinement may exceed 8 hours, and mortality increases as transport duration increases (Warriss et al., 1990). Temperature extremes are a major stressor for broilers during transport (Mitchell et al., 1992). Both hot and cold transport are associated with an increase in the physiological markers of poor welfare, as well as mortality (Nijdam et al., 2004; Nijdam et al., 2005). Some indicators of stress which increased during transport, reviewed by Mitchell and Kettlewell (1998), include corticosterone, HLR, glucagon, and creatine kinase. However, the stress caused by the thermal environment alone, especially in turkeys, has not been well distinguished.

Behavioural responses can give some indication of the magnitude of a thermal stressor, though they are also difficult to clearly relate to welfare status. Poultry will behaviourally thermoregulate in addition to their compensatory physiological processes (Strawford et al., 2011). Stocking density is an important determinant of how successful these responses will be. Decreasing stocking density in the trailer (to a degree) allows birds to move around, huddle, or space themselves to meet temperature demands (Delezie et al., 2007), improving their ability to withstand CBT disruption (Strawford et al., 2011). Not only will birds shift away from the coldest areas, they will also hide their head and feet to reduce exposed surface area, huddle in groups, ruffle their feathers (via ptiloerection) to increase insulation, burrow under cage mates, and shiver (Dawson and Whittow, 2000; Strawford et al., 2011; Schwartzkopf-Genswein et al., 2012). Peripheral vasoconstriction will also occur as the bird attempts to keep CBT constant (Strawford et al., 2011). At higher densities, behavioural thermoregulation is more difficult, but

the risk of injury may be decreased (Delezie et al., 2007). Though heat stress elicits compensatory behaviours such as panting and skin exposure, and can have negative health and welfare consequences, broilers do not consistently show aversion to high exposure temperatures alone (Abeyesinghe et al., 2001; Warriss et al., 2005).

2.5 Bird Effects

The broiler or turkey's external conditions are not the only determinant of how well it copes with thermal stress during transport. Aside from metabolic and behavioural compensations, the particular birds' inherent characteristics affect its ability to compensate for non-thermoneutral conditions. Genetics, age, sex, and size, as well as the prior temperature to which the bird had acclimatized, will impact the result of extreme transport conditions. The health condition of and any pre-existing pathologies in transported birds will also impact their ability to cope with transport.

Genetics. In broilers, lineages with a higher potential growth rate were more likely to experience ascites after cold stress as their oxygen demand exceeds their ability to supply (Deeb et al., 2002). These same lineages were also found to have more severely reduced weight gain after heat stress in the study by Deeb et al. (2002). The researchers also noted the moderate heritability for ascites-related traits, though modern birds have not been selected for ascites resistance. The age of chicken and turkey hens at the time of laying can also influence the body weight of her future offspring. A study by Huff et al. (2007) found that younger turkey hens produced lighter poults, who also tended to have higher blood glucose and heart glycogen levels. Broiler chickens from such hens are hypothesized to have a reduced inflammatory response, and potentially decreased thermoregulation abilities (Yalcin et al., 2005).

Age and Size. The age of the birds, which is often closely related to their size, impacts their ability to withstand both heat and cold stress. Younger birds are more negatively affected by cold temperatures, and small, 1.8-kg broilers can experience severe hypothermia when exposed to conditions below -8°C. In contrast, the larger, older, 2.6-kg birds can withstand temperatures as low as -14°C (Dadgar et al., 2011; Watts et al., 2011). Metabolic effects aside, the larger birds have a smaller surface area to mass ratio from which to lose heat (Watts et al., 2011). In addition

to the effects of size, older birds may be more feathered, and thus better insulated, than those from a younger flock. The study by Dadgar et al. in 2011 also found greater live shrink in younger birds after cold transport, though this can be partially explained by the larger proportion of body weight made up by the (now empty) GI tract, whereas in larger birds the GI tract accounts for a smaller percentage of total body weight. These smaller, more severely affected broilers also had a higher incidence of DFD meat, and higher pH after cold transport (Dadgar et al., 2012). During times of heat stress, the mechanisms beneficial to withstanding cold temperatures become disadvantageous – larger birds produce more heat, and have a smaller surface area to mass ratio from which to lose it (MacLeod and Hocking, 1993; Watts et al., 2011). Smaller broiler chicks, which had undergone a period of feed restriction earlier in life, resisted the effects of heat stress more robustly than those fed ad libitum, with improved survivability and lower HLR (Zulkifli et al., 2000). Among turkeys, sex determines age (and size) at transport, as they are reared separately. In Canada, the larger toms are typically transported at up to 17 weeks of age, and hens around 12 weeks, while in the USA, transport of Hybrid Converter toms and hens may occur at around 22 and 18 weeks of age, respectively (Turkey Farmers of Canada, 2017; Hybrid Turkeys, 2017).

Sex. The influence of sex on the performance of thermally-stressed poultry can result in significant differences in coping abilities of hens and toms. Male broilers seem to be at greater risk for transport stress than females, and make up a larger portion of the DOAs (Whiting et al., 2007). Male broilers were also found to have a lower CBT than females after cold exposure, despite their larger size, and the larger increase in muscle pH in response to pre-slaughter stress in male birds further indicates a heightened stress reaction (Dadgar et al., 2011; Strawford et al., 2011). The degree of feather coverage, more advanced in females than males of some broiler lineages, and the greater abdominal fat reserves may help explain the advantage of hens in cold conditions (Dawson and Whittow, 2000; Dadgar et al., 2011). A difference in percent live shrink between the sexes was not detected by Strawford et al. (2011).

Background. One additional factor which can affect birds' ability to thermoregulate are the conditions to which the bird was acclimatized. This can also apply to thermal conditioning when the birds are less than a week old. In broiler chicks conditioned to warm temperatures,

initiation times for thermoregulatory behaviours are shorter once they reach adulthood (Tanizawa et al., 2014). Plasma corticosterone, rectal temperature, and mortality were decreased in heat-experienced chickens (Yahav and Hurwitz, 1996). Cold-conditioning young broiler chicks for brief (1.5 to 3h) periods to temperatures as low as 5°C improved their ability to recover from subsequent cold exposure (Shinder et al., 2007). Thus, improving thermo-tolerance to expected transport conditions may provide a useful avenue of research for helping broilers and turkeys cope with extreme temperatures.

2.6 Managing Stressors

The stressors experienced during transport can have significant effects on welfare and meat quality. Potential stressors include catching and handling, feed and water withdrawal, and of course the actual transport. Birds experiencing thermal distress in the transport environment are being subjected to an additional, and sometimes very severe stressor. Certain management practices and other modifications to the transport environment can reduce the stress endured at this time, improving welfare and reducing economic loss. Adjusting loading density to account for bird characteristics as well as the external conditions can improve their ability to thermoregulate, but it is far from the only strategy. Holding birds in thermoneutral temperatures during lairage is also an important consideration, as higher ambient temperatures are associated with greater mortality (Whiting et al., 2007). Ensuring birds are in good health for transport is also beneficial, particularly that they are dry and have sufficient body condition. For heat-stressed birds, strategies such as water misting and expedited unloading would likely reduce mortality and condemnations. Care should also be taken during catching and loading, as this is a particularly stressful event, and injuries sustained will affect birds' ability to cope during their journey (Whiting et al., 2007; Vosmerova et al., 2010; Voslarova et al., 2007).

Trailer design, external temperatures, and the resulting microclimate are the most important determinants of whether or not the flock experiences thermal stress. Improved trailer design which includes active ventilation would offer more temperature control and increase uniformity throughout the trailer, but North America is slow to adopt this technology when, during hot transport, passive ventilation is adequate during motion (Kettlewell et al., 1993; Schwartzkopf-Genswein et al., 2012). Cold exposure is more difficult to control, and birds on the periphery of a load are likely to experience much lower temperatures than those in the hot thermal core

(Knezacek et al., 2010). Additionally, the occurrence of cold stress, particularly in turkeys, has not been well-characterized. This multifaceted transport process remains to be fully understood, a necessity in reducing the negative physiological and welfare consequences associated with transport stress.

3.0 METHODOLOGY

3.1 Experimental Design

3.1.1 Treatment Design

Three flocks of 40 hens and three flocks of 40 toms were used throughout the experiment, for an overall total of 240 birds (120 females, 120 males), split among five temperature and humidity treatments: 28°C with 80% relative humidity (RH), 28°C with 30% RH, 20°C with 80% RH, 20°C with 30% RH, and -18°C with humidity uncontrolled but typically ranging between 80% and 100% RH. The experimental design was a completely randomized design (CRD), with birds randomly distributed into treatments upon acquisition. The effects of cold transport (experiment 1) were examined via a one-way ANOVA using the cold (-18°C) and both moderate (20°C) treatments (which served as controls). The effects of warm transport (experiment 2) were analysed via a two-way ANOVA with a 2x2 factorial comparison of temperature and humidity, using data from the two moderate and two warm (28°C) treatments. The data from the moderate (20°C) treatments was shared between the two experiments. The data were analyzed using SAS 9.4 statistics software, using the mixed model procedure; further detail is provided within the materials and methods section of each manuscript. Behavioural data were log-transformed for normal distribution before analysis was conducted, with normality confirmed using the Shapiro-Wilk test. Treatments were compared within each sex as described, and between-sex comparisons were also made for each treatment condition using a T-test.

3.1.2 Birds and housing

All procedures and housing were approved by the U of S Animal Care committee. Forty Hybrid Converter turkeys (1 flock) were split into five pens (one per treatment) via random selection on arrival, and the eight turkeys in each treatment were split between two crates on the treatment day. Approximately five extra turkeys were acquired with each flock to replace any individuals found unfit for the trial due to injury or illness. Hens were approximately 12 weeks of age at the time of slaughter, and toms were 16 weeks of age, which were within the typical market age ranges for the Canadian turkey production industry. All hens were obtained from one producer, and all toms were also from a separate single producer. The turkeys were acquired

approximately one week before they reached the intended testing age to allow them to acclimate to new surroundings before undergoing simulated transport.

Hens were housed exclusively in an unused barn on the University of Saskatchewan (U of S) campus, in pens of 8 birds each. Sixteen birds, plus the spare birds, were kept in a larger pen, but all turkeys were housed at very low density: hen density was approximately 6 kg/m^2, and tom density was around 12 kg/m^2, well below the Codes of Practice (section 3.5) maximum stocking density of 55kg/m^2 (National Farm Animal Care Council, 2016). One of the tom flocks was kept in the Animal Care Unit on the University of Saskatchewan campus, in a single large pen, still well below maximum stocking density. Any injured or sick toms were moved to a separate but non-isolated pen to reduce further injury due to aggression and pecking, and were not used in the study. All birds were provided *ad libitum* access to clean drinking water and a complete feed obtained from their farm of origin. Lighting intensity was approximately 5 lux for all birds, with 16 hours on and 8 hours off. The dark period was set to a continuous 8 hours for the hens, but for the toms it was split into two 4-hour blocks with one hour of lights-on between them. The lighting programs were structured to match the hens' and toms' farms of origin. The temperature in the housing unit was between 13°C and 16°C. Turkeys were checked twice daily by a member of the research team.

3.1.3 Environmental Chamber

Two 2.1m by 3.4m climate-controlled chambers were used throughout the experiment, located in the Engineering building of the University of Saskatchewan. One chamber was capable of maintaining temperatures of up to approximately 40°C, while the second chamber could maintain temperatures as low as -25°C. Calibration data were collected on the chambers before the experiment was conducted to ensure the correct temperatures were being reached, in addition to the live temperature and humidity data used to make adjustments while the experiment was running. During the experiment, each crate was equipped with four miniature temperature and humidity data loggers, with two additional data loggers at the front and rear of the chamber. Conditions were also monitored in real time with the use of a temperature and a humidity sensor affixed to each crate, visible in Figure 3.1. Chamber temperature data indicated that the warm and moderate exposure conditions were stable. Though there was a brief (less than

30 minute) deviation from the set-point of up to 8°C immediately after loading, temperatures stabilized to within 3°C of the set-point for the remainder of the simulated transport events (Figure 3.2).

Figure 3.1: Eight hens crated in the chamber during 1 replication of the 20°C 80% RH treatment.

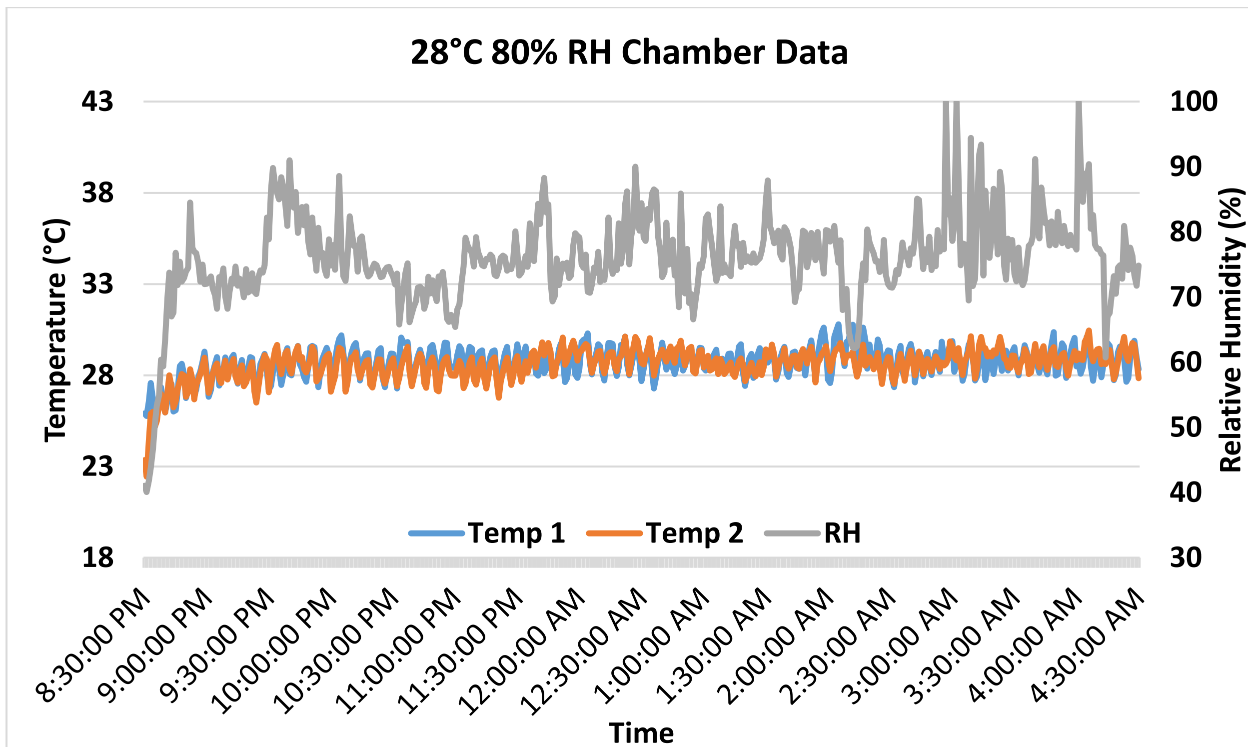


Figure 3.2: Temperature and humidity data for the duration of one replicate of the 28°C 80% RH exposure condition.

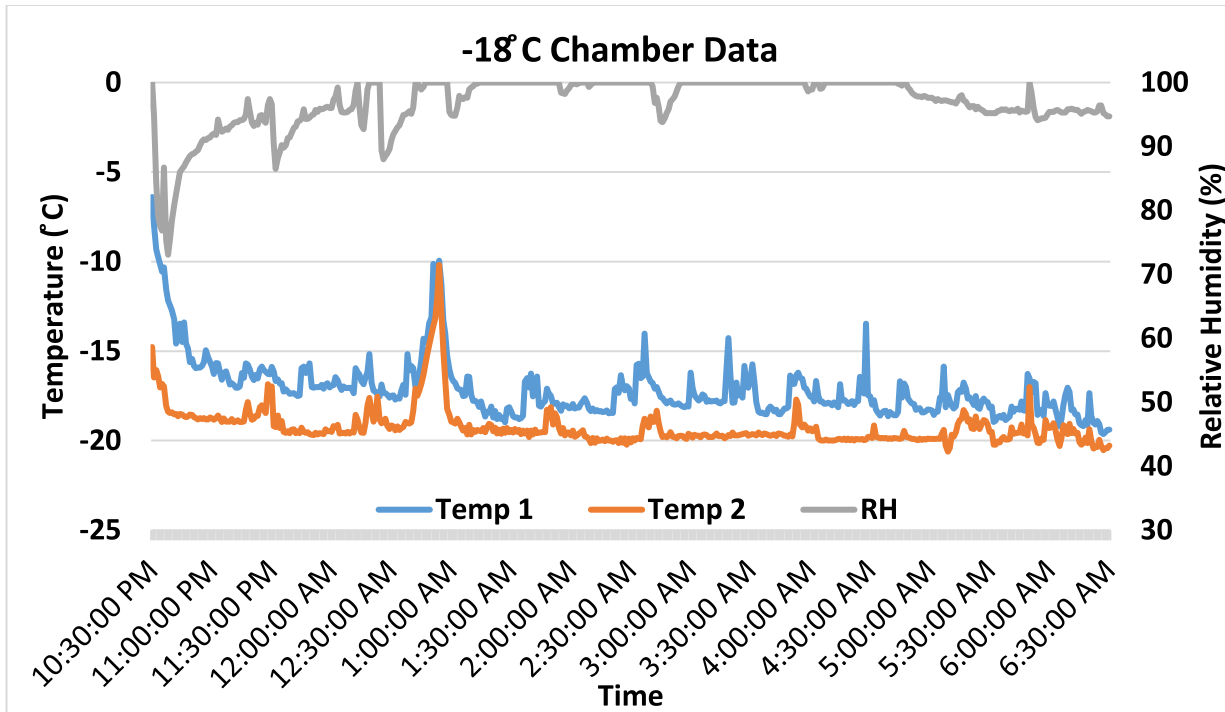


Figure 3.3: Temperature and humidity data for the duration of one replicate of the -18°C exposure condition.

The cold chamber conditions (-18°C) were punctuated by occasional defrost cycles, typically only once per replicate. Despite this, the temperature deviation did not exceed 10°C from the set-point in the cold treatments (except during the half hour period immediately after loading the birds), and conditions remained within 3°C of the set-point for the majority of the exposure period (Figure 3.3). Chamber humidity data, as in Figures 3.2 and 3.3, revealed that in the 20°C and 28°C treatments RH typically remained within a range of 10% higher or lower than intended, and at no point exceeded a deviation of 25% or greater. As mentioned, the relative humidity was uncontrolled in the -18°C treatment, and generally remained between 80% and 100%, but dropped as low as 20% at times.

A possible minor confounding effect of air speed, slightly higher in the cold (0.1 m/s) than warm chamber (0 m/s), was not able to be controlled. Both of the chambers were used in simulating moderate (control) conditions. Lighting intensity at bird level ranged from 15 to 30 lux in both of the chambers, sufficient to allow for behaviour monitoring.

3.2 Data Collection

Raw physiological and meat quality data for each bird are available in Appendix A. Data collection procedures are illustrated in Figure 3.4.

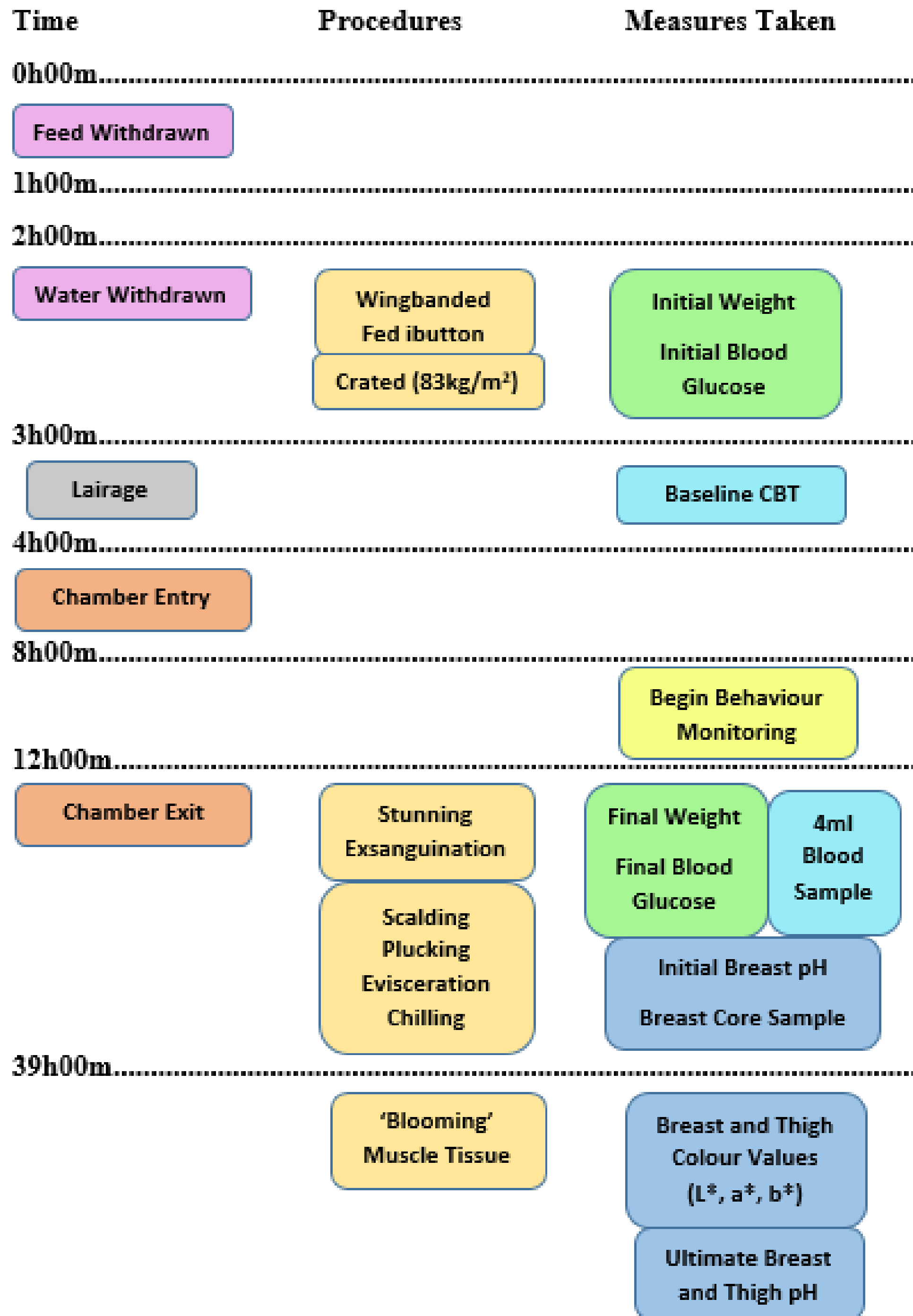
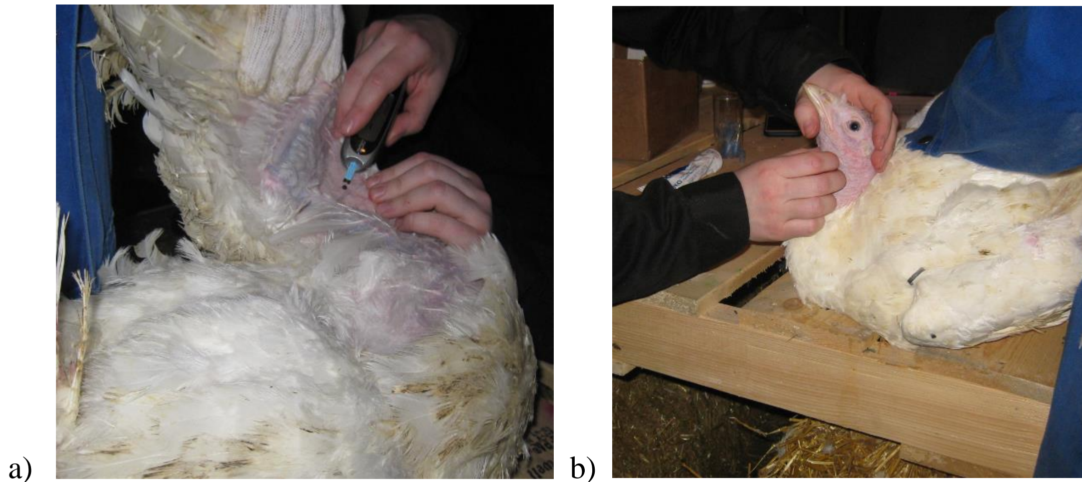


Figure 3.4: Data collection procedures and measures taken.

3.2.1 Pre-treatment Procedure and Measurements

Feed was withdrawn two hours prior to capture and four hours prior to the initiation of simulated transport, resulting in a total of 12 hours of feed withdrawal at the time of slaughter. Water was provided up until the point of capture and pre-treatment procedures, 10 hours prior to slaughter. Each bird was captured and handled in accordance with the humane bird handling techniques recommended in Sections 6.1 and 7.3 of the Codes of Practice (National Farm Animal Care Council, 2016). A cervical dislocation euthanasia device was on-hand in case a bird was found to be injured or suffering. The time was noted for each measurement during all phases of the experiment. Upon capture, some of the toms were fitted with a hood to reduce struggling and self-injury. Birds were weighed on a digital hanging scale (50# digital scale, Berkley, Columbia, SC), wing-banded (one on each wing), and assigned an identifying number. The average weight prior to testing was 7.4kg for hens and 16.4kg for toms. A blood glucose measurement was taken via a needle prick to a wing vein and analyzed by blood glucose meter (OneTouch UltraMini, LifeScan, Milpitas, CA, US) with the corresponding test strips (OneTouch Ultra 25, LifeScan, Milpitas, CA, US), as seen in Figure 3.5a. Birds were painted with an identifying marker using livestock paint in order to track their behaviour throughout the treatment. Turkeys were then given a randomly selected pre-calibrated miniature data logger (DS1923-F5#, Maxium Integrated, San Jose, CA) orally, shown in Figure 3.5b, which was recovered from the gizzard (ventriculus) or crop at the time of evisceration, to record internal body temperature throughout the treatment. CBT data during the last hour of exposure to treatment conditions were compared to baseline CBT data collected during the 1-h lairage period to determine ΔCBT.



a) b)

Figure 3.5: a) Blood glucose sample was taken using OneTouch glucose meter from the wing vein; b) Turkey hen with visible wingband is given an ibutton data logger which was often seated in the gizzard.

Each bird was placed into one of two numbered crates, with four birds per crate for a density of 83 kg/m^2 as is typical in a commercial setting. Crates for 16-week-old toms (expected weight of 16.5kg each) were approximately 0.42m high, 0.74m wide, and 1.07m long, while crates for 12-week-old hen (expected weight of 8.23kg each) were approximately 0.38m high, 0.54m wide, 0.74m long (Hybrid Turkeys, 2017). Each wheeled crate was fitted with four randomly selected USB data loggers (Lascar EL-USB-2-LCD+ data logger, Lascar Electronics, Erie, PA, US) to record temperature and humidity at bird level, where the data loggers were secured with zip ties in mesh bags. Birds were transported approximately 5 minutes via a partially enclosed trailer to the Hardy lab in the Engineering building on the U of S campus. Upon arrival, birds were allowed to rest quietly in a darkened room for one to one and a half hours prior to treatment. Two batches of eight birds were processed each day, staggered by two hours.

3.2.2 Treatment Procedure

Two hours after initial capture, and after approximately one to one and a half hours of rest or lairage at 13 to 15°C, birds were moved via their crates into a climate-controlled chamber located in Room 1B39 of the Engineering building. Conditions within each of two chambers were controlled for temperature and humidity and equipped with live video feed to observe bird

condition and behaviour throughout the treatment and for later analysis. Positions of the crates within the chamber were recorded. Each group of eight birds per flock was subjected to one of the five treatments. Exposure time for each treatment was eight hours. Turkeys were supervised for the duration of the treatment. After exposure, birds were immediately moved via crates to Room 1A33 of the Engineering building for final measurements, slaughter, and processing.

3.2.3 Post-treatment Measurements and Slaughter Procedure

Each bird was identified by wingband number. Glucose measurement and weight were taken in the same manner as during the pre-treatment procedure, using the same equipment. Live shrink loss was calculated as a percentage by subtracting final weight from initial weight and dividing by initial weight, then multiplying by 100%. Birds were hung on turkey-specific shackles on a purpose-built steel bar, four at a time. Birds were stunned to induce unconsciousness with an electric stunning knife (VS200, Midwest Processing Systems, Minneapolis, MN, USA) on power setting 5 (circa 0.16 amps, 60 Hz AC) for 30 seconds, or until the wing-droop response or cessation of nictitating membrane response was observed by the stunner. Birds were immediately exsanguinated by severing the jugular vein, and a blood sample of approximately 4 ml was obtained at this time. A 5 ml EDTA (Ethylenediamine tetraacetic acid) anti-coagulation tube was used, and sample tubes were capped, rinsed, and inverted several times to prevent clotting before being placed on ice. Contamination between birds was unlikely, as each tube was opened just prior to sample collection and capped immediately after. The head of each bird was removed prior to scalding at 68°C for 30 seconds to 1 minute (when tail and wing feathers were easily pulled out) and mechanical plucking. Each bird was scalded and plucked individually or in pairs, rinsed, and eviscerated. Wingband numbers were verified prior to taking initial meat quality measurements.

3.2.4 Meat Quality Measurements

Sample collection, pH probe. Immediately after evisceration, typically 15 minutes post-mortem, a 5-g breast muscle core sample was obtained from the ventral, upper-left breast (pectoralis major) using metal coring tubes. These core samples were wrapped in numbered foil,

flash frozen in liquid nitrogen to halt muscle metabolism, and transferred to -80°C storage for subsequent initial pH testing using the slurry method, as described by Stewart et al. (1984). At this time a small slit was made in the skin near where the core sample was obtained to allow the insertion of a pH electrode calibrated at pH 4.0 and 7.0 (Accumet, Fisher Scientific, Ottawa, ON, Canada) with a portable pH meter and accompanying temperature probe (Hanna H1 9025 microcomputer pH meter, North Highlands, CA, US). This pH measurement was identified as the initial breast muscle pH. The temperature probe was inserted into the breast muscle near the calibrated pH probe but did not come in contact with it. The probe was held as still as possible in an upright position and the reading was allowed to stabilize before recording. The probe was rinsed with distilled water after each use and wiped with a lab wipe (Kim-Tech Kimwipes, Kimberly-Clark, Irving, TX, US) when necessary, and was recalibrated between treatments (every 8 birds). Processed carcasses were packed in ice singly or in pairs within rubber totes and refrigerated at 4°C.

Slurry pH method. Approximately 24 to 27 hours post-mortem, after chilling, a second pectoralis major muscle core sample was taken for analysis of ultimate pH, shown in Figure 3.6. Both the initial and ultimate core samples, previously frozen in liquid nitrogen and stored in -80°C, were analyzed to verify breast muscle pH according to the slurry method. Samples were removed from freezer storage in the Agriculture Building room 4C19 approximately 5 minutes before being processed to facilitate uniform mixing, but no portion was allowed to thaw completely. The same pH probe as previous was used, and was calibrated prior to processing, and recalibrated as needed. Five grams (+/- 0.05g) of breast tissue was obtained from the centre of the core sample and finely diced. The sample was placed in 20 ml of distilled water in a plastic 50-ml sample tube and homogenized at 14.5k rpm for 30 seconds. If sufficient sample was not available, 1 g of breast tissue was processed with 10 ml of distilled water. Immediately after the sample had been homogenized, the pH probe was inserted into the sample tube and the reading recorded once it had stabilized.

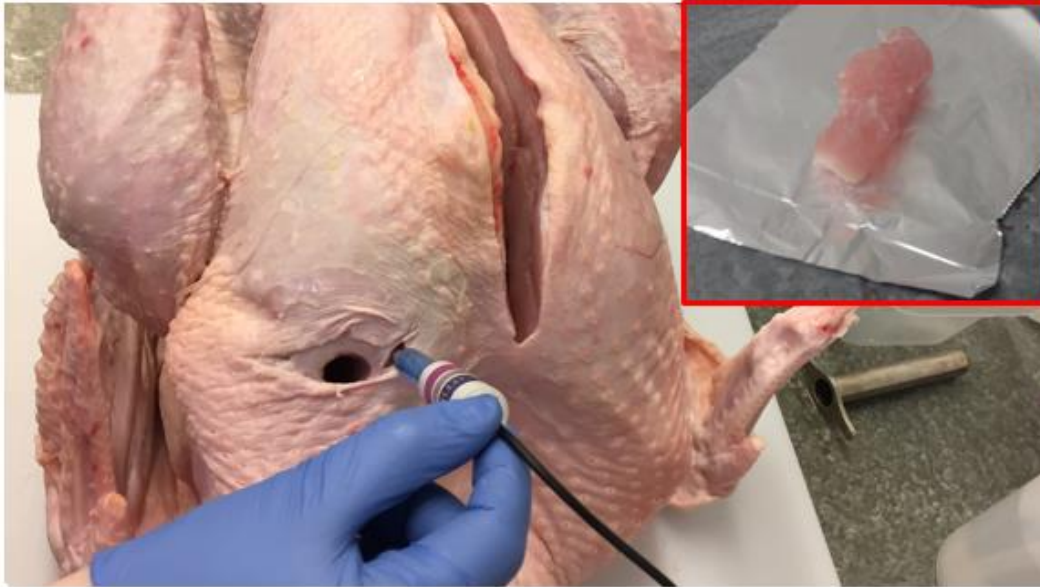


Figure 3.6: Breast pH probe measurement and breast core location at 27 hours post-mortem, after colour readings were obtained. Inset: breast core sample for pH slurry.

Colour readings. At 24 to 28 hours post-mortem, the thigh and upper right breast muscles were allowed to 'bloom' by parting the skin and making a long incision in the breast muscle, which was sliced vertically from top to bottom to expose the inner portion between the major and minor pectoralis muscles. The skin ventrally joining the thigh to the body was sliced to allow the femur to lay flat against the table on the same plane as the bird's back. The process of blooming occurs as the exposed muscle tissue binds oxygen from the air to tissue myoglobin, forming oxymyoglobin and stabilizing the colour in line with consumer expectations and industry practice (Young and West, 2001). After 30 minutes of blooming, a colour measure was obtained from the right breast muscle and thigh using a Minolta colour meter (RC-400, Minolta, Ramsey, NJ, US) and included SpectraMagic NX software, demonstrated in Figure 3.7. Two readings from each muscle were obtained, the second reading after rotating the meter 90 degrees, to account for differences due to muscle fibre direction. Each reading was assigned an identifying number which was recorded. The colour readings obtained were converted by the software included and used to determine a*, b*, and L* colour values (redness, yellowness, and lightness). Following the colour reading, a second core sample was taken near to the location of the first core on the left breast muscle, following the same procedure as for the first core.

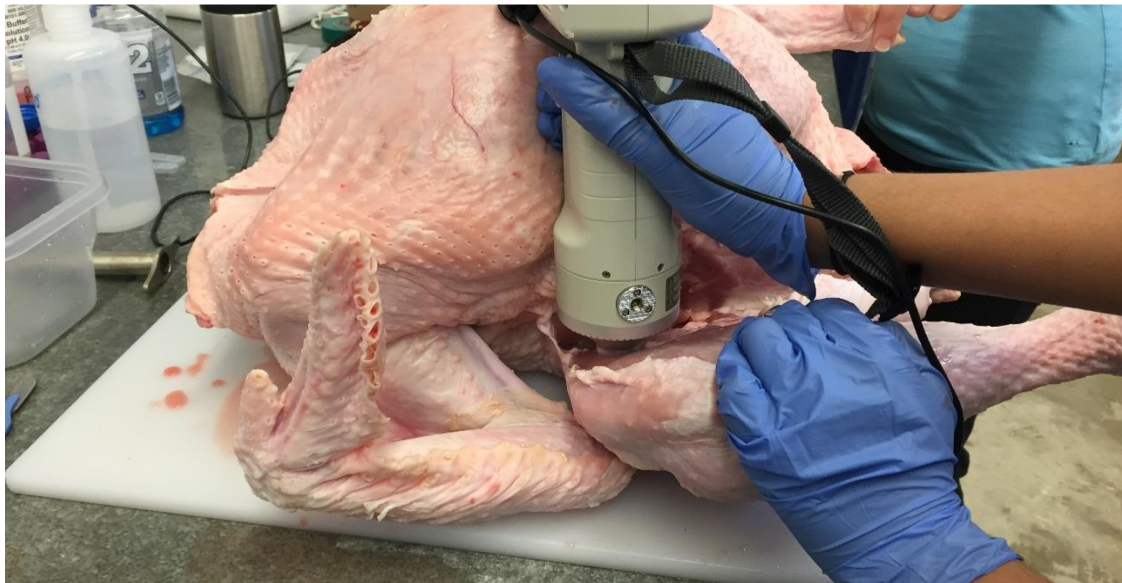


Figure 3.7: Thigh colour value measurement location with Minolta colour meter.

3.2.5 Blood Smear Preparation and Staining

The 4ml blood sample collected in the 5-ml EDTA tubes at the time of slaughter was transported on ice to room 4C19 in the Agriculture Building immediately after all 8 samples from a treatment had been collected. The tubes were promptly placed on a blood tube roller or mixer rack to prevent clotting. A small drop of blood was transferred to a clean, labelled glass slide using a heparinized capillary tube. Two blood smears for each sample were made manually using the two slide wedge method. The smears were allowed to dry completely for at least 48 hours before staining with Ricca Wright-Giemsa stain and Giordano buffer solution. Smears were stained using the Wright-Giemsa staining procedure supplied by the manufacturer and allowed to air-dry before being stored in slide boxes (Ricca Chemical Company, 2005). To determine the heterophil/lymphocyte ratio (HLR), the slides were viewed at 1000x magnification using an oil immersion lens. The microscope was focused on an area of the slide where cells were distributed evenly without overlapping each other, and the first 100 leukocytes observed were differentiated. The HLR of these 100 was determined by dividing the number of heterophils by the number of lymphocytes, shown in Figure 3.8. Each of the two slides for each bird's blood sample was counted three times. Final HLR were compared, but no baseline data were collected prior to treatment.

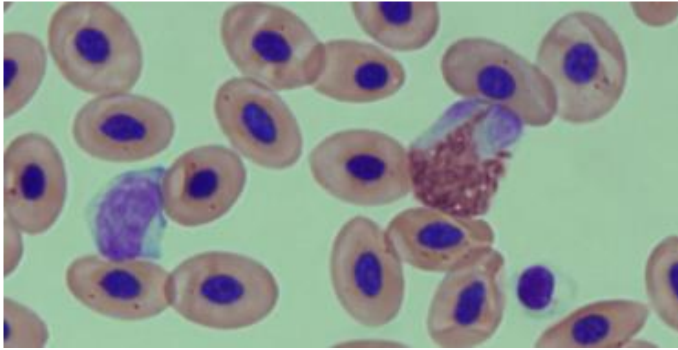


Figure 3.8: A lymphocyte (left) and heterophil (right) in the domestic turkey.

3.2.6 Behaviour Analysis

Data were obtained by instantaneous scan sampling conducted every 5 minutes for the last 4 hours of recorded video and audio. The last 4 hours were selected to capture behaviour that might indicate if thermal stress had developed under typical transport durations in the specified exposure conditions. Up to 5 seconds of recorded video before and after each sampling point was viewed to determine exactly what was occurring at the sampling point (Lehner, 1998). This sampling frequency provided 49 observations for each pen (replicate unit). Behaviours were recorded as a number of birds in each crate performing the behaviour at that moment in time (from 1 to 4 per crate, with each of the 49 observations including 8 bird-actions total). All behaviour data was recorded twice, by two separate observers, and the resulting time budgets were averaged. Data validation was performed between the two observers via a paired comparison of one replicate per treatment in SAS 9.4. Proc TTest was used to compare datasets, which did not differ significantly ($p > 0.05$). Each behaviour recorded is defined in Table 3.1, with some definitions adapted from Webster A.B. (2000). All behaviours except panting were mutually exclusive, and separate categories were created for combinations of behaviours and position (sitting or standing) to allow for more detailed data collection – categories were later combined based on statistical significance and relevance. Raw time budget data containing uncombined categories are available in Appendix B.

Table 3.1: All of the different behaviours observed during the study and their defining criteria. Behaviour categories were divided based on position (standing or sitting), and all were mutually exclusive with the exception of panting (Webster, 2000).

Behaviour	Definition
Still	The bird is motionless
Active	The bird is standing or attempting to rise, and moving feet or wings, changing position, or changing location in the crate
Sit at Rest	Bird is in the sitting position, motionless with its body contacting the floor of the crate
Survey	Quick head movements in an alert bird, suggesting visual surveillance of the environment
Peck	The beak is used to peck at other birds or objects, including sensors and the floor of the crate
Huddle	Birds are grouped closely in an area of the crate, with minimal movement OR birds are actively attempting to 'burrow' beneath or between other birds
Preen	The beak is used to comb through or manipulate any area of feathers on the bird's own body
Shiver	The wings or body of the bird quiver repeatedly
Ptiloerection	The feathered skin covers the bare neck, feathers are ruffled
Pant	The bird breathes through an open beak (not mutually exclusive)
Head Rest	The head is rested heavily on the floor or slats of the crate, or another bird, panting may occur but the bird does not otherwise stand or move
Skin Expose	The unfeathered skin of the neck is exposed, feathers are spread to expose bare skin of the torso, or wings are drooped.

4.0 SIMULATED COLD TRANSPORT

The research work completed as part of this thesis involved exposing turkeys to cold temperatures (-18°C), with uncontrolled humidity, and moderate (20°C) and warm (28°C) temperatures with both low (30%) and high (80%) relative humidity levels. Their responses to cold conditions and warm conditions were compared to those at control (moderate) conditions, which were shared between both experiments. The following manuscript examines the effects of cold exposure with uncontrolled, high relative humidity during simulated transport on market-age turkey hens and toms, in order to increase understanding of their response to such conditions and the acceptable thresholds of transport temperature. Physiological measures, meat quality indicators, and behaviour observations allow for inferences about productivity and welfare to be made in an effort to expand the body of knowledge on both the transport process and the less often studied domestic turkey. This manuscript has been submitted to, but not yet published by, the Poultry Science journal and may be subject to copyright restrictions.

Many hard-working individuals were involved with data collection for this project and with providing guidance on methodology. As first author, my role in the preparation of the following manuscript included analysis and presentation of the findings. My supervisor Dr. Trever Crowe served as reviewer, editor and corresponding author. Catherine Vermette also had a role as a reviewer and editor of the manuscript, and played a major part in organization of the project. Dr. Karen Schwean-Lardner offered much of her expertise in statistics and poultry science, with an emphasis on ethology, in addition to reviewing the manuscript. Further review was conducted by my advisory committee members Dr. Hank Classen and Dr. Fiona Buchanan. The draft citation for the manuscript is as follows:

Henrikson, Z.A., Vermette C.J., Schwean-Lardner K., and Crowe, T.G. 2016. Effects of simulated cold transport on physiology, meat quality, and behavior of turkey hens and toms. *Awaiting publication* in Poultry Science.

4.1 Abstract

The impact of cold exposure during simulated transport was assessed in 12-wk-old turkey hens and 16-wk-old toms. Turkeys (72 toms, 72 hens) were randomly divided into 3 male and 3 female groups: two moderate 20°C groups with either 30% or 80% relative humidity (RH) and a cold group exposed to -18°C, with uncontrolled, high RH. Groups of 8 birds (one replicate unit) were observed in a climate-controlled chamber for 8h prior to slaughter. Core body temperature (CBT), live shrink, heterophil/lymphocyte ratio (HLR), and change in blood glucose levels were assessed; meat quality measures included thigh and breast muscle pH and L*, a*, and b* colour values. Significance was declared at $p \leq 0.05$. Live shrink in hens exposed to -18°C (2.8%) was greater ($p = 0.001$) than those in the 20°C treatments (1.5%). No differences in CBT, blood glucose, or HLR were detected, though tendencies were noted ($p \leq 0.10$). Thigh pH was higher in the -18°C treatment (hens: 6.39; toms: 6.08) than in both 20°C groups. Colour values (L*, a*, and b*) were measured 27h post-mortem. In the -18°C exposed hens, breast L* values were lower, and thigh a* and breast b* values were higher than in both 20°C treatments. No differences were detected in colour values among toms. Behaviour differences were noted between treatments; more time was spent huddling, shivering, preening, and with feathers ptiloerected in cold-exposed turkeys. Hen and tom responses to cold exposure were compared, revealing higher live shrink in hens (hens: 2.8%; toms: 1.5%), and a larger decrease in blood glucose in toms (hens: 2.51mmol/L; toms: 2.65mmol/L). Meat characteristics differed between sexes, with higher initial breast pH (hens: 6.72; toms: 6.47) and ultimate thigh pH (hens: 6.39; toms: 6.08), and lower breast b* in hens exposed to -18°C. Cold exposure resulted in higher live shrink, darker meat with greater redness, and a tendency for blood glucose to decrease, with differences in the magnitude and type of effects experienced between sexes.

4.2 Introduction

In North America, almost all commercial livestock will be transported at least once during their lives. This event can be a significant stressor which may threaten welfare, and is an area of economic loss due to dead on arrivals (DOA), condemnations, and live shrink loss. Production of poultry occurs year-round, and a variety of inclement transport conditions may be experienced, including severe cold in Canada and the northern US. While the domestic turkey,

Meleagris gallopavo, is considered susceptible to thermal stress at high temperatures, little research exists on the effects of cold exposure.

Attempts to protect birds from cold external temperatures during transport via tarps can lead to reduced ventilation within the trailer, which can result in high humidity levels, a central core of high temperatures, and the periphery still only a few degrees warmer than the external temperature (Knezacek et al., 2010; Burlinguette et al., 2012). Cold exposure can result in dark, firm and dry meat (DFD) (Mallia et al., 2000; Owens et al., 2000), a defect not well received by consumers. Previous research on the effects of cold exposure on poultry during transport has been limited in extent, and almost entirely restricted to broiler chickens. While some inferences may be made from broiler research, turkeys differ in their size and age at transport, as well as in their feathering and physiology. Nonetheless, cold transport conditions in broilers have been found to result in several significant effects.

Core body temperature (CBT), around 40.3 to 40.8°C in turkeys, has a positive relationship with exposure temperature (Mills et al., 1999; Yahav et al., 2008; Strawford et al., 2011). Broilers are considered hypothermic when CBT drops below 39.3°C according to Richards (1977), with a CBT of approximately 22°C proving fatal (Sturkie, 1946). Wet broilers were more susceptible to cold stress than their dry comparators and were found to experience a drop in CBT at temperatures around +8°C, while dry birds could withstand -4ºC without any decrease in CBT (Hunter et al., 1999). Additionally, the younger, smaller, 1.8-kg broilers were found to experience more severe hypothermia when exposed to temperatures near -8°C, while older 2.6-kg birds tolerated temperatures as low as -14°C (Dadgar et al., 2011; Watts et al., 2011). Broiler and broiler breeder mortality has been noted to increase when birds are transported during colder months (October through April) in the Czech Republic, where mean ambient temperature ranged from 7°C to -3°C for the 7-year period (Vecerek et al., 2006; Voslarova et al., 2007). Declining CBT in cold-stressed broilers has also been correlated with blood glucose decreases, and the accompanying depletion of muscle glycogen reserves suggest there is a lack of energy available for sufficient thermoregulation (Dadgar et al., 2011; Dadgar et al., 2012).

Cold exposure may also result in changes in meat quality in broilers, including increased muscle pH, with darker and sometimes redder meat, though the effects on colour and tenderness are not conclusive (Lee et al., 1976; Boulianne and King, 1998; Dadgar et al., 2010; Chan et al.,

2011; Dadgar et al., 2011). Greater water holding properties in turkey and broiler meat (Dadgar et al., 2010; Dadgar et al., 2011; Strawford et al., 2011), along with larger yield, reduced drip and cook loss, and improved texture and taste scores for white meat (Fernandez et al., 2002) may also accompany these biochemical changes. Excessively dark meat with a high pH might have the DFD (dark-firm-dry) defect, and odor and shelf life may be negatively affected (Allen, Russel, and Fletcher, 1997; Allen et al., 1998). The point at which meat quality has been observed to decline most noticeably is at exposure temperatures below -14ºC, where DFD incidence reached 60% in immobilized birds (Dadgar et al., 2011). Cold conditions during transport have also been demonstrated to increase live shrink (Dadgar et al., 2011), but other contrary evidence attributes this to the stress of transport (and the accompanying compulsory feed withdrawal) alone (Nijdam et al., 2005; Aviagen, 2009).

Stress and welfare are also influenced by cold exposure and thermal stress in general. Both hot and cold transport are associated with an increase in physiological markers of poor welfare, including higher mortality (Nijdam et al., 2004; Nijdam et al., 2005). An elevated heterophil-lymphocyte ratio (HLR) has been used as an indicator of chronic environmental, social, and thermal stressors (Gross and Siegel, 1983; Maxwell, 1993; Zhang et al., 2009). Heat stress has been demonstrated to produce an increase in the HLR in broilers (Mitchell and Kettlewell, 1998; Altan et al., 2003; Zhang et al., 2009), and though the effects of cold transport have not been directly measured, a study by Gross (1988) determined that exposure to cool housing temperatures of 6°C for 1 day results in higher HLR ratios.

The effects of cold exposure during transport of turkeys have not been determined, despite the potential for detriment to productivity or welfare. To gain an understanding of turkey responses to conditions typical of winter transport, the objectives of this research were to determine the effects of cold exposure on:

1. selected physiological and behavioural indicators of thermal stress and welfare, and

2. selected indicators of turkey meat quality.

4.3 Materials and Methods

All procedures and housing were approved by the University of Saskatchewan Animal Care Committee's Animal Research Ethics Board.

4.3.1 Experimental Design

Three flocks (replicates) of 24 turkey hens each and 3 flocks of 24 turkey toms were used throughout the experiment, for a total of 72 hens and 72 toms. Each replicate of 24 birds was split into 3 treatment groups consisting of 8 birds (in 2 crates of 4 each) exposed for 8 hours to 1 of the following conditions: 20°C with 80% relative humidity (moderate-temperature, high-humidity group), 20°C with 30% relative humidity (moderate-temperature, low-humidity group), or -18°C with uncontrolled humidity, typically ranging between 80-100% relative humidity (cold-temperature group). The experimental design was a completely randomized design, with birds randomly distributed into 1 of the 3 treatment groups.

The comparison between the cold and both moderate (20°C) temperature groups (which served as controls) consisted of a multiple treatment comparison of the 3 treatments, with the replicate unit being a pen (8 birds). The data were analyzed using SAS 9.4 statistics software (SAS 9.4, Cary, NC, USA), using the mixed model procedure. The model was $Y = \mu + T + e$, where Y is the dependent variable, μ is the population mean of the variable, T is the treatment effect (fixed), and e is the random error. A one-way analysis of variance was used to make comparisons, and DDFM KenwardRoger was used to approximate degrees of freedom. Means separation was performed using Tukey's studentized range test, and significance was declared at $p \leq 0.05$, trends at $p \leq 0.10$. A comparison was also performed between sexes within the same treatment condition via a T-test, with a model of $Y = \mu + G + e$, where G is gender. Percentage data from behaviour observations were log-transformed (log+1) to achieve a normal distribution (confirmed with the Shapiro-Wilk test for normality) before significance was tested.

4.3.2 Birds and Housing

All hens were obtained from 1 producer, and all toms from a separate single producer, approximately 1 week before they reached the intended testing age to allow them to acclimate to the new environment before undergoing 8 hours of simulated transport. The 24 Hybrid Converter turkeys comprising 1 replicate were randomly split into 3 pens (1 per treatment) on arrival, and the 8 turkeys in each treatment were split between 2 crates on the treatment day. Hens were approximately 12 weeks of age at the time of slaughter, and toms were 16 weeks of age.

Turkeys were housed at very low densities (6 to 12 kg/m^2) in either an unused barn or in the Animal Care Unit on the University of Saskatchewan campus. All birds were provided *ad*

libitum access to clean drinking water and a complete feed obtained from their farm of origin. Lighting intensity was approximately 5 lux for all birds, with 16 hours of light and 8 hours of dark per 24-h period. The lighting programs chosen were structured to match the hens' and toms' farms of origin, with hens exposed to a continuous 8-hour dark period and toms exposed to two 4-hour dark periods with 1 hour of light between them. The temperature in both housing units was between 13°C and 16°C.

4.3.3 Environmental Chamber

Two 2.1m by 3.4m climate-controlled chambers were used throughout the experiment, located in the Engineering building of the University of Saskatchewan. During the experiment, each crate was equipped with 4 USB temperature and humidity data loggers (EL USB 2+, Lascar Electronics Inc., Erie, PA, USA), with 2 additional data loggers at the front and rear of the chamber. Conditions were also monitored in real time with the use of a thermocouples and a humidity sensor (HM1500LF, Measurement Specialities, Inc., Impasse Jeanne Benozzi, France) affixed to each crate. Temperature deviation from the set point did not exceed ±5°C in the moderate-temperature treatments, and ±8°C in the cold treatments. Humidity in the moderate treatments remained within a range of 10% higher or lower than intended throughout the exposure period, excluding the first 15 minutes as the chamber stabilized. As mentioned, the relative humidity in the -18°C treatment was generally between 80% and 100%, but dropped as low as 20% when the door to the chamber was opened to allow inspection of the birds approximately once every two hours. Air speed was low in both chambers (0-0.1 m/s), and light intensity at bird level ranged from 15 to 30 lux, sufficient to allow for behaviour monitoring.

4.3.4 Data Collection

Pre-treatment Procedure and Measurements. Feed was withdrawn 2 hours prior to capture and 4 hours prior to the initiation of simulated transport, resulting in a total of 12 hours of feed withdrawal at the time of slaughter. Water was provided up until the point of capture and pre-treatment procedures. Birds were weighed on a digital hanging scale (BTDFS50-1, Berkley, Columbia, SC, USA), wing-banded (one numbered band on each wing), and assigned an identifying number. Average weight prior to exposure was 7.4kg for hens and 16.4kg for toms. Blood glucose was measured via a needle prick to a brachial vein and analyzed by a blood glucose meter (OneTouch UltraMini, LifeScan, Milpitas, CA, US). Birds were orally

administered a miniature data logger (DS1923-F5#, Maximum Integrated, San Jose, CA), which was recovered from the gizzard or crop at the time of evisceration, to record internal body temperature throughout the treatment. Baseline CBT (average during 1-h lairage period) was compared to mean CBT during the last hour of treatment. Finally, birds were marked with livestock paint in order to track their behaviour throughout the treatment.

Birds were placed into 1 of 2 sex-specific crates, 4 birds per crate for a density of 83 kg/m^2. Each crate was fitted with 4 data loggers (Lascar EL-USB-2-LCD+ data logger, Lascar Electronics, Erie, PA, US) to record temperature and humidity at bird level. Birds were transported in a partially enclosed trailer approximately 1.5 km from their holding site to the Engineering building. Birds were then given a lairage period in a quiet, darkened room for 1 to 1.5 hours prior to treatment, at between 13°C and 15°C.

Treatment Procedure. 2 h after initial capture, the crates holding the birds were moved into 1 of the 2 climate-controlled chambers. The chambers were equipped with video feed to observe bird condition and behaviour throughout the treatment and for later analysis. After the 8-h exposure, birds were immediately moved via crates to the processing room for final measurements, slaughter, and processing.

Post-treatment Measurements and Slaughter Procedure. Blood glucose concentrations and live weight were recorded according to the same procedures used during the pre-treatment measurements. Birds were hung on turkey-specific shackles and stunned with an electric stunning knife (VS200, Midwest Processing Systems, Minneapolis, MN, USA) for 30 s on power setting 5 (circa 0.16 amps, 60 Hz AC), or until the wing-droop response or loss of nictitating membrane reflex was observed by the stunner. Birds were immediately exsanguinated by severing the jugular vein, and a blood sample of approximately 4 ml was collected in EDTA (Ethylenediamine tetraacetic acid) anti-coagulation tubes and placed on ice. Birds were scalded at 68°C for 30 seconds to 1 minute (when wing and tail feathers could easily be pulled out), mechanically plucked, and manually eviscerated. Initial meat quality measurements were then recorded.

Meat Quality Measurements. Immediately after evisceration, an initial post-slaughter 5-g breast muscle core sample was obtained from the ventral, upper-left pectoralis major (breast). Core samples were flash frozen in liquid nitrogen and transferred to -80°C storage until muscle

pH testing using a slurry method (Stewart et al., 1984). Breast muscle pH was also measured near the core sample collection site using a pH probe (Accumet, Fisher Scientific, Ottawa, ON, Canada) with a portable pH meter and accompanying temperature probe (Hanna H1 9025 microcomputer pH meter, North Highlands, CA, US). Carcasses were then packed in ice singly or in pairs within rubber totes and refrigerated at 4°C. Approximately 24 to 27 hours post-mortem, a second core sample was extracted from each carcass for determination of ultimate pH, as shown in Figure 4.1.

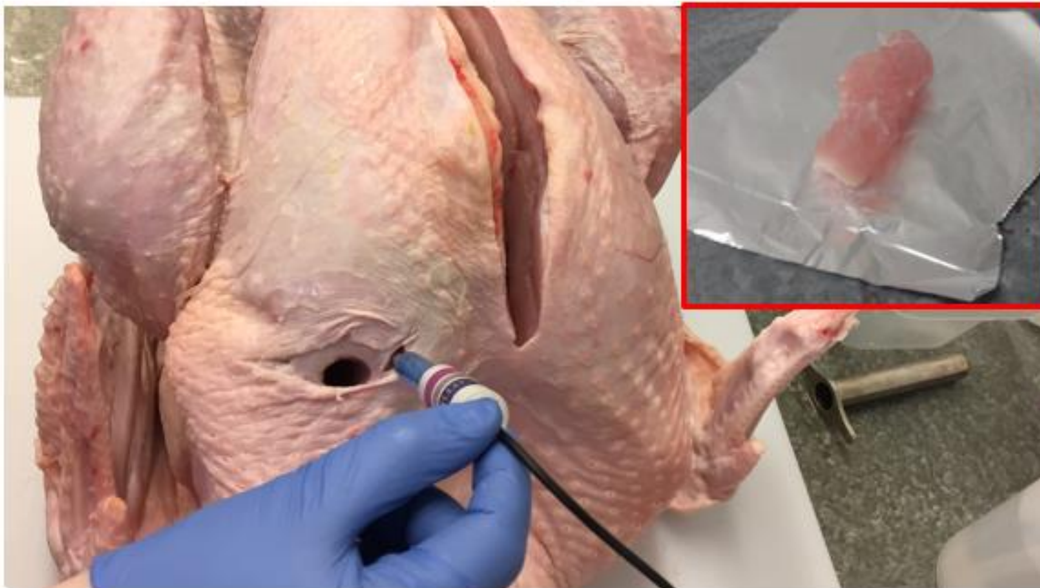


Figure 4.1: Breast pH probe measurement and breast core location at 27 hours post-mortem, after colour readings were obtained. Inset: breast core sample for pH slurry.

At 24 to 27 hours post-mortem, the thigh and upper right pectoralis muscles were allowed to 'bloom' by parting the skin and making a long vertical incision in the breast muscle, exposing the inner portion between the major and minor pectoralis muscles. The skin ventrally joining the thigh to the abdomen was sliced to allow the femur to lay flat against the table on the same plane as the bird's back. After 30 minutes of exposure to air, a colour measure was obtained from the right breast muscle and thigh using a Minolta colour meter (RC-400, Minolta, Ramsey, NJ, US), as in Figure 4.2. Two readings from each muscle were obtained, the second reading after rotating the meter 90 degrees, to account for differences due to muscle fibre orientation. The colour readings were converted to a*, b*, and L* colour values (redness, yellowness, and lightness).

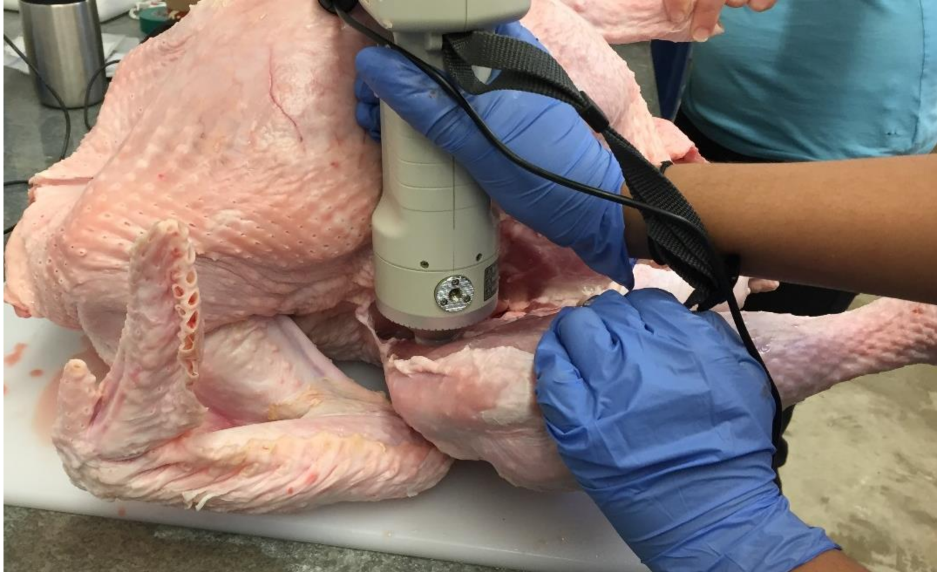


Figure 4.2: Location of thigh colour value measurement with Minolta colour meter.

Previously collected breast muscle core samples were processed by first removing them from freezer storage to partially thaw. Approximately 5 g of breast tissue from the centre of the core sample was finely diced, placed in 20 ml of distilled water and homogenized at 14.5k rpm for 30 seconds (Polytron PT-3100, Kinematica, Lucerne, Switzerland). Immediately after homogenization, the pH was measured, using the electronic probe.

Blood Smear Preparation and Staining. A small drop of blood was transferred from each EDTA tube to a glass slide and smeared manually using the two slide wedge method. The smears were allowed to dry before staining with Ricca Wright-Giemsa stain and Giordano buffer solution. Smears were stained using the Wright-Giemsa staining procedure supplied by the manufacturer and allowed to air-dry before being stored in slide boxes (Ricca Chemical Company, 2005). To determine the heterophil/lymphocyte ratio (HLR), slides were viewed and photographed at 1000x magnification using an oil immersion lens. The first 100 leukocytes were differentiated, and the HLR of these 100 was determined by dividing the number of heterophils by the number of lymphocytes. Three counts were performed per slide and averaged.

4.3.5 Behavioural Analysis

Data were obtained by instantaneous scan sampling at 5-minute intervals for the last 4 h of recorded video. Up to 5 seconds of observations before and after each sampling point was

viewed to determine exactly what was occurring at the sampling point (Lehner, 1998). Behaviour were recorded as the number of birds within each of the 2 crates performing a particular behaviour at that moment in time, with pen (containing 8 birds) serving as the replicate unit. All video data were observed by 2 observers to quantify the behavioural activity, and the resulting time budgets were averaged. Data validation was performed on 1 replicate of each treatment (for both males and females) in SAS 9.4 as a paired comparison, using Proc TTest to compare the datasets of the 2 observers – datasets were not significantly different. Each behaviour recorded is defined in Table 4.1, with some definitions adapted from Webster (2000). Behaviours were mutually exclusive with the exception of panting, and separate categories were devoted to combinations of behaviours to allow for more detailed data collection.

Table 4.1: All of the different behaviours observed during the study and their defining criteria. Behaviour categories were divided based on position (standing or sitting), and all were mutually exclusive with the exception of panting (Webster, 2000).

Behaviour	Definition
Still	The bird is motionless
Active	The bird is standing or attempting to rise, and moving feet or wings, changing position, or changing location in the crate
Sit at Rest	Bird is in the sitting position, motionless with its body contacting the floor of the crate
Survey	Quick head movements in an alert bird, suggesting visual surveillance of the environment
Peck	The beak is used to peck at other birds or objects, including sensors and the floor of the crate
Huddle	Birds are grouped closely in an area of the crate, with minimal movement OR birds are actively attempting to 'burrow' beneath or between other birds
Preen	The beak is used to comb through or manipulate any area of feathers on the bird's own body
Shiver	The wings or body of the bird quiver repeatedly
Ptiloerection	The feathered skin covers the bare neck, feathers are ruffled
Head Rest	The head is rested heavily on the floor or slats of the crate, or another bird, panting may occur but the bird does not otherwise stand or move
Pant	The bird breathes through an open beak (not mutually exclusive)

4.4 Results and Discussion

4.4.1 Physiological Measures

Live shrink, blood glucose decrease, change in CBT, and the HLR are shown for moderate and cold-exposed turkey hens and toms in Tables 4.2 and 4.3, respectively. Exposure to -18°C for an 8-h period had significant impact on the physiology, meat quality, and behaviour of turkeys undergoing simulated transport, with more pronounced effects in the smaller and younger female turkeys than in males. Hens exposed to the -18°C treatment had a tendency ($p = 0.07$) toward a larger decrease in CBT (-0.61°C) than hens in the 20°C 30% RH treatment, where CBT increased slightly from baseline by 0.18°C. As CBT is homeostatically (and behaviourally) maintained constant, significant deviation from baseline may indicate that thermoregulatory mechanisms have been overwhelmed; however, the magnitude of the effect experienced by hens in these conditions is not likely to pose a severe health or welfare issue on its own. These results contrast broiler chicken research, where similar, and even less extreme cold temperatures elicited a significant decrease in CBT – the larger size and greater age of turkeys likely plays a role in this difference (Dadgar et al., 2011; Strawford et al., 2011; Watts et al., 2011). The core body temperatures of toms exposed to -18°C did not differ from those in either 20°C treatment.

In comparison to the 1.5% live shrink loss experienced by turkeys exposed to either of the 20°C treatments, hens exposed to -18°C had a higher ($p = 0.001$) mean live shrink of 2.8%. The significantly higher live shrink experienced by cold-exposed hens suggests that transport in these conditions required greater energy expenditure. While all transported birds experience some live shrink due to deprivation of feed and water, the increased energy demands of thermoregulation in a cold environment can be substantial (Schwartzkopf-Genswein et al., 2012). This additional loss agreed with research on the effects of cold exposure in broilers (Dadgar et al., 2011) and is worthy of attention from both a welfare and productivity perspective – while shrink loss has not been decisively tied to negative welfare, it has a clear impact on the economic value of birds received at the abattoir.

Table 4.2: Physiological and meat quality measures taken in turkey hens exposed to 20°C 30% RH, 20°C 80% RH, and -18°C and the p-values for significant differences and trends.

Measure	Treatment			P-Value	SEM
	20°C 30% RH	20°C 80% RH	-18°C		
Live Shrink (%)	1.45b	1.46b	2.79a	0.001	0.234
ΔGlucose (mmol/L)	-2.29	-1.65	-2.52	NS	0.214
ΔCBT (°C)	0.18[1]	-0.08	-0.61	0.070	0.175
HLR	1.54	1.60	1.68	NS	0.086
Initial Breast pH (slurry)	6.52	6.56	6.72	0.077	0.039
Initial Breast pH (probe)	6.73	6.66	6.67	NS	0.028
Ultimate Breast pH (slurry)	5.64	5.65	5.73	NS	0.023
Ultimate Breast pH (probe)	5.71	5.69	5.77	NS	0.021
Ultimate Thigh pH (probe)	5.93b	5.91b	6.39a	0.005	0.085
Thigh L*	51.27	51.02	48.39	0.060	0.591
Thigh a*	13.13b	13.49b	14.94a	0.018	0.322
Thigh b*	0.90	0.88	0.08	NS	0.239
Breast L*	51.08a	51.29a	49.05b	0.048	0.454
Breast a*	4.89	4.80	4.57	NS	0.093
Breast b*	-2.93b	-2.96b	-3.80a	0.011	0.160

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens (8 birds/replication), except CBT for the 20°C 30% RH treatment (average response of 16 hens).
[CBT] Core Body Temperature.
[HLR] Heterophil Lymphocyte Ratio.

Table 4.3: Physiological and meat quality measures taken in turkey toms exposed to 20°C 30% RH, 20°C 80% RH, and -18°C and the p-values for significant differences and trends.

Measure	Treatment			P-Value	SEM
	20°C 30% RH	20°C 80% RH	-18°C		
Live Shrink (%)	1.61	1.47	1.54	NS	0.122
ΔGlucose (mmol/L)	-0.59	-1.32	-2.65	0.076	0.398
ΔCBT (°C)	-0.27	0.09	-0.45	NS	0.156
HLR	1.82	1.33	1.70	NS	0.126
Initial Breast pH (slurry)	6.45	6.43	6.47	NS	0.033
Initial Breast pH (probe)	6.73	6.76	6.68	NS	0.031
Ultimate Breast pH (slurry)	5.70	5.71	5.72	NS	0.013
Ultimate Breast pH (probe)	5.71	5.69	5.73	NS	0.009
Ultimate Thigh pH (probe)	5.82b	5.87b	6.08a	0.008	0.046
Thigh L*	50.20	49.70	48.85	NS	0.392
Thigh a*	12.80	13.90	11.11	NS	0.976
Thigh b*	-0.41	0.46	-0.85	NS	0.493
Breast L*	50.39	50.01	49.95	NS	0.397
Breast a*	6.40	5.52	8.10	NS	1.018
Breast b*	-2.20	-2.58	-1.31	NS	0.282

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[CBT] Core Body Temperature
[HLR] Heterophil Lymphocyte Ratio

In toms exposed to -18°C, neither live shrink nor CBT were different from those exposed to 20°C, indicating that this level of cold exposure did not considerably compromise their live weight productivity or energy status. Male turkeys exposed to -18°C did however have a tendency ($p = 0.0764$) toward a larger decrease in blood glucose than those in the 20°C 30% RH treatment, with average decreases of 0.6 mmol/l and 2.7 mmol/l, respectively. Blood glucose concentrations may have some value in indicating changes in metabolic status, and past research has shown that it generally decreases after lengthy transport and the accompanying feed withdrawal (Nijdam et al., 2005), but this measure was not clearly and consistently affected by exposure to -18°C. In hens, no differences in blood glucose levels between treatments were detected. No differences were detected between turkeys exposed to the -18°C and both 20°C treatment conditions, in hens nor toms.

Between-sex comparison revealed that live shrink varied significantly among male and female turkeys within the -18°C treatment, with a mean shrink loss of 2.8% in hens and 1.5% in toms (Table 4.4). The lack of difference between sexes in both 20°C treatments suggests the difference after cold exposure was due a differing response to the lower temperature, and not differences in response to transport in general.

The magnitude of decrease in blood glucose concentration varied between toms and hens across all treatments. Toms had a larger drop in blood glucose than hens, with 2.65 and 2.51 mmol/L decreases, respectively, in the -18°C treatment ($p=0.001$). However, at 20°C the drop in blood glucose concentration was greater in hens than in toms: 2.29 vs. 0.59 mmol/L at 20°C 30% RH and 1.65 and 1.32 mmol/L in the 20°C 80% RH treatment. Despite hens experiencing greater live shrink during cold exposure, they had a smaller drop in blood glucose than the toms, whose live shrink was not significantly affected. The implication of the inconsistent changes in blood glucose levels between the sexes is not clear, but may be due to inherent differences in physiology between hens and the larger, older toms. HLR and magnitude of change in CBT did not differ significantly between hens and toms within any treatment condition.

Table 4.4: All physiological measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, and -18°C and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Live Shrink (%)				
-18°C	2.79a	1.54b	0.002	0.289
20°C 30% RH	1.45	1.61	NS	0.134
20°C 80% RH	1.46	1.47	NS	0.162
ΔGlucose (mmol/L)				
-18°C	-2.51b	-2.65a	0.001	1.184
20°C 30% RH	-2.29a	-0.59b	0.006	0.688
20°C 80% RH	-1.65a	-1.32b	0.016	0.740
ΔCBT (°C)				
-18°C	-0.61	-0.45	NS	0.108
20°C 30% RH	0.35[1]	-0.27	NS	0.299
20°C 80% RH	-0.08	0.09	NS	0.100
HLR				
-18°C	1.68	1.70	NS	0.135
20°C 30% RH	1.54	1.82	NS	0.129
20°C 80% RH	1.60	1.33	NS	0.119

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens or toms (8 birds/replication), except CBT for the 20°C 30% RH treatment (average response of 16 hens, 24 toms).
[CBT] Core Body Temperature
[HLR] Heterophil Lymphocyte Ratio

4.4.2 Meat Quality Indicators

Initial and Ultimate pH. Hen initial and ultimate pH measures are shown in Table 4.2. There was a tendency (p = 0.0767) for hens exposed to -18°C to have a higher initial breast pH (6.72) than hens exposed to 20°C 30% and 80% RH, where pH was 6.52 and 6.56, respectively. The ultimate thigh pH (probe method) of hens also differed significantly (p = 0.0045), with a mean pH of 6.39 in those exposed to -18°C compared to 5.93 and 5.91 in the 20°C 30% and 80% RH treatments, respectively. The tendency for cold-exposed hens to have a higher initial breast pH is in line with previous research on the effects of cold exposure on broiler meat pH, as is the increase in ultimate thigh pH (Lee et al., 1976). These differences indicate that changes in muscle pH were occurring promptly after slaughter, and persisted after chilling in the thigh meat, though no significant differences were detected in ultimate breast pH.

Ultimate thigh pH differed in the toms, with birds in the -18°C treatment having a higher ($p = 0.0082$) mean pH of 6.08, compared to 5.82 and 5.87 in birds exposed to 20°C 30% RH and 20°C 80% RH, respectively (Table 4.3). No other differences in muscle pH were detected. In combination with the measured physiological differences, this finding further supports the notion that males withstood the effects of cold exposure more robustly than females. Despite this, minor differences in the ultimate meat quality still arose in toms subjected to cold-temperature transport.

Colour Values. Meat colour in hens was affected by cold temperature exposure (Table 4.2). Thigh meat a* was significantly higher (more red) in hens exposed to -18°C, with a value of 14.9 compared to 13.1 and 13.5 in the 20°C 30 % and 80% RH exposed hens. The b* (yellowness) value was lower in the breast meat of cold-exposed hens, with a mean b* value of -3.8 in the -18°C treatment compared to -2.93 and -2.96 in the 20°C 30 % and 80% RH treatments, respectively. Hen thigh ($p = 0.0602$) and breast ($p = 0.0564$) meat also had a tendency to be darker, with lower L* values, in the -18°C exposed hens than in those exposed to 20°C 30 % and 80% RH. No colour differences were observed between toms in the 3 treatments (Table 4.3).

While the evaluation of meat quality is important from a consumer and processing standpoint, it also gives additional information on the biological response of turkeys to thermal stressors. Colour values (L*, a*, and b*) and pH data serve as indicators of changes in the metabolic state of the tissue, and have been correlated with various product quality characteristics such as drip loss, cook loss, toughness, and shelf life (Fernandez et al., 2002; Allen et al., 1997). The darker and more red-coloured, high-pH meat observed in hens was consistent with the effect of cold exposure on broilers, and suggests that some alterations to muscle metabolism had occurred (Lee et al., 1976; Dadgar et al., 2010; 2011). Decreases in muscle pH and changes in colour values after cold exposure occur as a result of reduction in ante-mortem and post-mortem muscle glycolysis, which if extensive, can predispose to DFD (dry-firm-dark) meat traits. These changes are not generally detrimental to quality or processing, but associated colour differences may impact consumer acceptance (Froning et al., 1978; Fletcher, 1999; Mallia et al., 2000).

Between-sex Comparison. Several pH differences were also noted between hens and toms within the same treatment condition (Table 4.5). After exposure to -18°C, the initial pH of breast meat (slurry method) was higher in hens (6.72) than in toms (6.47), as was ultimate thigh pH, with a mean of 6.39 in hens and 6.08 in toms. No differences in initial breast pH (slurry or probe) were detected between hens and toms in the 20°C treatments. Ultimate breast pH (slurry), however, was higher in toms (5.71 vs. 5.65) in the 20°C 80% RH treatment, a finding which was unexpected.

Table 4.5: All initial and ultimate pH measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, and -18°C and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Initial Breast pH (slurry)				
-18°C	6.72a	6.47b	0.024	0.064
20°C 30% RH	6.52	6.45	NS	0.048
20°C 80% RH	6.56	6.76	NS	0.044
Initial Breast pH (probe)				
-18°C	6.67	6.68	NS	0.028
20°C 30% RH	6.73	6.73[1]	NS	0.058
20°C 80% RH	6.66	6.76	NS	0.036
Ultimate Breast pH (slurry)				
-18°C	5.73	5.72	NS	0.026
20°C 30% RH	5.64	5.70	NS	0.022
20°C 80% RH	5.65b	5.71a	0.031	0.017
Ultimate Breast pH (probe)				
-18°C	5.77	5.73	NS	0.021
20°C 30% RH	5.71	5.71[1]	NS	0.002
20°C 80% RH	5.69	5.69	NS	0.020
Ultimate Thigh pH (probe)				
-18°C	6.39a	6.08b	0.017	0.076
20°C 30% RH	5.93	5.83[1]	NS	0.036
20°C 80% RH	5.91	5.87	NS	0.043

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens or toms (8 birds/replication), except initial breast pH (probe), ultimate breast pH (probe), and ultimate thigh pH (probe) for the 20°C 30% RH treatment, which includes the average response of 16 toms and 24 hens.

Table 4.6: All meat colour measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 30% RH, and -18°C and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Thigh L*				
-18°C	48.39	48.85	NS	0.503
20°C 30% RH	51.27	50.20	NS	0.482
20°C 80% RH	51.02	49.70	NS	0.524
Thigh a*				
-18°C	14.94	11.11	NS	1.442
20°C 30% RH	13.13	12.80	NS	0.566
20°C 80% RH	13.49	13.89	NS	0.556
Thigh b*				
-18°C	0.19	-0.85	NS	0.595
20°C 30% RH	0.90	-0.41	NS	0.406
20°C 80% RH	0.88	0.46	NS	0.478
Breast L*				
-18°C	49.05	49.95	NS	0.601
20°C 30% RH	51.08	50.39	NS	0.334
20°C 80% RH	51.29	50.01	NS	0.479
Breast a*				
-18°C	4.57	8.10	NS	1.579
20°C 30% RH	4.89	6.40	NS	0.624
20°C 80% RH	4.80b	5.52a	0.045	0.195
Breast b*				
-18°C	-3.80a	-1.31b	0.016	0.623
20°C 30% RH	-2.93	-2.20	NS	0.242
20°C 80% RH	-2.96	-2.58	NS	0.131

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).

Two differences in meat colour values (Table 4.6) were detected between the sexes. Breast b* was lower in hens exposed to -18°C, with a mean of -3.8 in toms and a mean of -1.3 in hens. Lower b* values indicate a weaker yellow (or stronger blue) hue. In the 20°C 80% RH treatment, the a* value of tom breast meat (5.52) was higher than that of hen breast meat (4.80), indicating greater redness. The pH and colour differences observed further supported the notion that 16-week-old toms are better able to resist the effects of cold exposure than smaller 12-week-

old hens, which showed more similar responses to cold-stressed broilers. The unexpected response of tom breast pH and colour to the warmer, high-humidity 20°C treatment may indicate some physiological or thermal stress is occurring, but could also be a result of inherent gender differences that are simply more apparent in these conditions.

4.4.3 Behavioural Analysis

Hens. Several differences in frequency and type of behaviours were observed between the time-budgets of hens exposed to -18°C and those exposed to the 20°C treatments (Table 4.7). Cold-exposed hens were observed to huddle together 51.7% of the time ($p < 0.0001$), preen (4.3%), shiver (5.9%), and ptiloerect their feathers (27.7%) more frequently ($p = 0.014$, $p < 0.0001$, and $p = 0.001$ respectively) than those in the 20°C conditions, and also spent less time standing and sitting still, 0.1% and 2.9% of time budgets, respectively ($p = 0.003$, $p < 0.0001$). In other words, cold-exposed hens favoured performing thermoregulatory behaviours over sitting or standing completely at rest. The huddling behaviour observed is thought to allow for reduced heat loss by limiting cold-exposed body surfaces, while shivering generates body heat directly through metabolic processes associated with muscle movement. Ptiloerection was likely employed to increase the insulative capacity of the feathers and further reduce radiant heat loss. This is consistent with past findings in broiler chickens, turkeys, and other avian species, which have demonstrated these heat-preserving behavioural responses to cold exposure (Dietz et al., 1997; Dawson and Whittow, 2000; Strawford et al., 2011). It is possible that preening was performed for some thermoregulatory benefit, though contrary evidence has found reduced preening in red jungle fowl in cold conditions, and the behaviour has also been linked to stress (Sherry, 1981; Kostal et al., 1992). While cold-exposed hens performed a wide range of thermoregulatory behaviours with greater frequency than those in neutral temperatures, these actions were not sufficient to completely avoid physiological effects. Cold-exposed hens did not appear to experience great physical or behavioural distress, but they may be nearing their limit of tolerable exposure conditions for the 8-hour duration which was evaluated.

Toms. The time budgets of toms (Table 4.7) also varied between treatments. Huddling behaviour occurred more often ($p = 0.003$) in the -18°C exposed birds, 30.1% of the time, as did ptiloerection (56.5%), shivering (2.2%), and preening (5.1%), which were observed rarely or not at all in the moderate temperature treatments ($p < 0.0001$, $p < 0.0001$, $p = 0.021$). The differences

in behaviour between treatments imply that despite the male turkeys' minimal physiological response to cold exposure, some behavioural modifications were necessary to avoid these effects. Toms in the 20°C 30% RH group also tended to spend more time active than those in the -18°C treatment (4.3% vs 1.6%, respectively; $p = 0.078$), a behaviour which may have been reduced in favour of huddling among the cold-exposed toms. Increased exercise has been demonstrated to increase CBT in quail exposed to both cold (0°C) and 1.5 m/s airspeed, but not cold and still air (<0.1 m/s) conditions (Zerba and Walsberg, 1992), such as those experienced by the toms.

Table 4.7: Grouped behaviour data (%) in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, and -18°C and the p-values and standard error. Measures are mutually exclusive, except panting.

	Behaviour	-18°C	20°C30%RH	20°C80%RH	P	SEM
Hens	Active	4.5	5.1	7.2	NS	0.53
	Stand Still	0.1b	2.3a	6.5a	0.003	1.28
	Sit at Rest	2.9b	86.5a	82.1a	<0.0001	13.70
	Huddle	51.7a	1.4b	0.3b	<0.0001	8.78
	Shiver	5.9a	0b	0b	<0.0001	1.03
	Ptiloerection	27.7a	0b	0b	0.001	5.05
	Preen	4.3a	1.3b	1.3b	0.014	0.59
	Survey	0.1	1.0	0.4	NS	0.20
	Peck	0.7	2.0	1.9	NS	0.26
	No Observation	2.1	0	0.3	NS	0.68
Toms	Active	1.6	4.3	2.4	0.078	0.56
	Stand Still	0	0.8	0.6	NS	0.19
	Sit at Rest	3.4	56.3	33.2	NS	9.91
	Huddle	30.1a	2.4b	0.4b	0.003	5.95
	Shiver	2.2a	0b	0b	<0.0001	0.37
	Ptiloerection	56.5a	0b	0b	<0.0001	10.03
	Preen	5.1a	0.4b	0.3b	0.021	1.10
	Survey	0.5	1.4	1.4	NS	0.38
	Peck	0.3	0.6	0.4	NS	0.10
	Head Rest	0b	3.8b	33.0a	0.002	6.29
	Pant	0	15.9	33.8	0.074	6.39
	No Observation	0	13.9	7.9	NS	3.53

Durations of the head-resting behaviour also varied between treatments, with higher frequencies observed with increasing temperature or humidity, and no occurrence at all in toms exposed to -18°C. Toms in the 20°C treatments also tended ($p = 0.074$) to pant more (33.8% of the time at 80% RH, 15.9% at 30% RH) than those in the -18°C treatment, which did not pant at all, indicating that even at 20°C toms employed heat-coping thermoregulatory behaviours. Time spent surveying and pecking (at other birds or the environment) were not significantly different between treatments.

Between-sex Comparison. Several differences in behaviour between hens and toms in the same exposure conditions were detected. As shown in Table 4.8, the -18°C exposed hens were active more often than toms, at 4.5 and 1.6% of their respective time budgets ($p = 0.019$). Shivering occurred more ($p = 0.028$) in cold-exposed hens than toms, 5.9 and 2.2% of the time, respectively. Hens were not observed to survey at all, a behaviour which toms engaged in 0.5% of time ($p = 0.010$). Conversely, hens tended to display ptiloerection less often than toms, 27.7% compared to 56.5% of the observed time period, respectively. These behaviour findings generally agree with the discrepancy between physiological responses of cold-exposed hens and toms, with the latter experiencing fewer effects of cold exposure (Watts et al., 2011).

In the 20°C 30% treatment, toms had a tendency to spend less time standing still than hens, 0.8 and 2.3% of the time, respectively. Toms also did not spend any time huddling at 20°C and 30% RH, while hens were huddled in 1.4% of observations ($p = 0.012$). Hens in the 20°C 30% RH group pecked significantly more often ($p = 0.011$) than toms, 2% and 0.6% of the time, respectively. Several of the same differences between hen and tom behaviour were observed in the 20°C 80% RH treatment. Toms again spent less time standing still than hens, 0.6 and 5.8% of the time ($p = 0.012$). They were also active less often than hens, with observed activity frequencies of 2.4 and 7.2%, respectively ($p = 0.015$). Again, pecking occurred more often in hens than in toms, 1.9 and 0.4% of their respective time budgets ($p = 0.024$). Leg strength and health of the older toms may play a role in differences in standing and activity levels compared to hens, though leg pathologies were not measured.

Table 4.8: Grouped behaviour data (%) in turkey hens and toms exposed to each treatment with p-values and standard errors. Measures are mutually exclusive, except panting.

	Behaviour	Hens	Toms	P	SEM
-18°C	Active	4.5a	1.6b	0.019	0.69
	Stand Still	0.1	0	NS	0.43
	Sit at Rest	2.9	3.4	NS	0.45
	Huddle	51.7	30.1	NS	8.09
	Shiver	5.9a	2.2b	0.028	0.96
	Preen	4.3	5.1	NS	1.20
	Ptiloerection	27.7	56.5	0.087	8.96
	Peck	0.7	0.3	NS	0.14
	Survey	0b	0.5a	0.010	0.13
	No Observation	2.1	0	NS	4.86
20°C 30% RH	Active	5.1	4.3	NS	0.54
	Stand Still	2.3	0.8	0.072	0.42
	Sit at Rest	86.5	58.7	NS	8.76
	Huddle	1.4a	0b	0.012	0.36
	Preen	1.3	0.4	NS	0.44
	Survey	1.0	1.4	NS	0.54
	Peck	2.0a	0.6b	0.011	0.34
	Head Rest	0b	3.8a	0.039	1.10
	Pant	0b	15.9a	0.006	4.77
	No Observation	0	13.9	NS	4.86
20°C 80% RH	Active	7.2a	2.4b	0.015	1.24
	Stand Still	5.8a	0.6b	0.012	1.56
	Sit at Rest	82.1	33.2	NS	13.46
	Preen	1.3	0.3	NS	0.37
	Survey	0.4	1.4	NS	0.31
	Peck	1.9a	0.4b	0.024	0.39
	Head Rest	0.1b	33.0a	0.002	9.13
	Pant	0.7	33.8	NS	15.60
	No Observation	0.3	7.9	NS	3.03

Moderate-temperature Treatments. Two additional behaviours occurred more often in toms exposed to 20°C 30% RH, which were not recorded at all in hens: panting during approximately 16% of observed time budgets (this behaviour was not mutually exclusive), which allows for evaporative cooling ($p = 0.006$), and resting of the head on the crate or other birds (3.8% of observed time budget, $p = 0.039$). This latter behaviour may be a posture of rest which allows for additional conductive heat loss from the un-feathered, exposed skin of the neck, rather than the more commonly observed head-tucked resting position (Buchholz, 1996). These behaviours were not expected at moderate control temperatures and suggested that 20°C was warmer than the thermoneutral range, particularly for the toms, which were indeed housed at approximately 15°C.

These noteworthy differences in behaviour associated with warm-temperature thermoregulation, as well as some differences in meat quality, were also detected in the toms exposed to 20°C and 80% relative humidity. Toms in this group tended to pant more frequently (33.8% of the time, $p = 0.074$) and displayed more head-resting behaviour (33%, $p = 0.002$) than toms in both the -18°C, where these behaviours did not occur, and more than in the 20°C 30% RH treatments where panting was observed 15.9% and head-resting 3.8% of the time. Hens demonstrated less than 1% frequency of these behaviours even at 20°C and 80% RH, though significant differences in panting were not detected between hens and toms in this condition, likely due to a high standard error. Nonetheless, these changes agree with the idea that thermoneutral temperatures for these toms had been exceeded, and heat-reducing behaviours were required to maintain homeostasis. Toms in this exposure group also had a significantly higher ultimate breast pH and a* (redness) colour value than hens in the same condition, differences which were not observed among the 20°C 30% treatments, but are also not clearly associated with warm exposure as reported in the literature. Both pH and a* have been generally demonstrated to decrease in heat-exposed poultry, though Askit et al. (2006) did observe an increased a* (Babji et al., 1982; Sams and Mckee, 1997; Dadgar et al., 2010). Though meat quality findings were somewhat inconsistent with past research, behaviour differences nonetheless suggest that toms experienced mild heat stress in the 20°C 80% RH exposure condition.

4.5 Conclusions

Physiology and meat quality measures were affected by exposure to -18°C in turkey hens, but the impact was not severe enough to greatly compromise meat quality. Hen CBT decreased, and live shrink increased, indicating that thermoregulatory mechanisms were reaching their limit, even with additional energy expenditure. In toms, the effects of cold exposure were less dramatic, with males experiencing minimal impacts on physiology and meat quality. The larger size (and associated decreased surface area from which to lose heat) of toms likely plays a significant role, but other factors, such as feathering and metabolic differences, must also be considered. The lack of clear results of cold exposure in blood glucose measurements impedes drawing conclusions, but all transported birds did experience a decrease as expected during times of energy expenditure, and cold exposure had a tendency to accentuate this effect in toms.

Previous studies have found significant effects of stressors in comparing a single measurement of HLR (Beuving et al., 1989), but no significant differences were detected between turkeys exposed to the -18°C and both 20°C treatment conditions, in hens nor toms. This may have been affected by the lack of baseline HLR values, which limited the usefulness of the data due to individual HLR variability. The lack of significance may also have been related to the relatively brief exposure period, as HLR changes do not develop immediately after stressors are initiated, and the degree of stress to which the birds were exposed. Despite the lack of significant difference in HLR between treatments, the differences in behaviour occurring with cold exposure suggest that hens were stressed under these conditions. While the impact on welfare is difficult to quantify, birds experienced discomfort, and a disruption and restriction of normal behaviour. The necessity of feed withdrawal during transport exposes birds to hunger and thirst, the severity of which may be affected by energy expended on thermoregulation. Toms showed similar responses to cold exposure as hens, with less shivering but more ptiloerection, suggesting their welfare was impacted to a similar degree.

The meat quality measures indicated that the immediate and 27-h post-mortem muscle physiology of hens had been altered by exposure to -18°C, with minor associated colour changes, which is worthy of consideration for processors but not likely to compromise the overall quality of the meat. Cold-exposed toms were largely unaffected. Because colour measures were only slightly impacted by transport in these conditions, consumer rejection of

products is not anticipated to occur on this basis. The limited changes in toms further indicate a more robust resistance to cold exposure than hens – but both sexes of turkey appear more cold-tolerant than broilers.

These results suggest that transport of turkeys at an exposure temperature of -18°C is not likely to cause major distress, but does result in discomfort and behaviour changes indicative of stress. While not extreme, welfare is decreased by exposure to these conditions. Furthermore, hen live shrink and some meat quality measures were compromised, which may affect productivity. Additional research is needed to further understand the effects of wind speed, colder temperatures, and humidity or moisture level during transport, as well as field research which can incorporate variations in trailer microclimate into our understanding of the effects of cold-temperature transport in turkeys.

5.0 SIMULATED WARM TRANSPORT

The research work completed as part of this thesis involved exposing turkeys to a cold temperature condition (-18°C), with uncontrolled humidity, and moderate (20°C) and warm (28°C) temperatures with low and high relative humidity (30% and 80%). Data collected for this research were subjected to two separate analyses comparing the responses to cold conditions and warm conditions to those at control (moderate) conditions, with the control condition data shared between both analyses. The following manuscript explores the impacts of warm exposure during simulated transport on market-age turkey hens and toms, in order to gain a more complete understanding of their response to transport conditions, as well as the acceptable thresholds of factors such as temperature and humidity. Physiological measures, meat quality indicators, and behavior observations allow for inferences about productivity and welfare to be made, in hopes of expanding the limited research on both the transport process and the less often studied domestic turkey. This manuscript has been submitted to, but not yet published by, the Poultry Science journal, and may be subject to copyright restrictions.

Many hard-working individuals were involved with data collection for this project and with providing guidance on methodology. As first author, my role in the preparation of the following manuscript included analysis and presentation of the findings. My supervisor Dr. Trever Crowe served as reviewer, editor and corresponding author. Catherine Vermette also had a role as a reviewer and editor of the manuscript, and played a major part in organization of the project. Dr. Karen Schwean-Lardner offered much of her expertise in statistics and poultry science, with an emphasis on ethology, in addition to reviewing the manuscript. Further review was conducted by my advisory committee members Dr. Hank Classen and Dr. Fiona Buchanan. The draft citation for the manuscript is as follows:

Henrikson, Z.A., Vermette C.J., Schwean-Lardner K., and Crowe, T.G. 2016. Effects of simulated warm transport on physiology, meat quality, and behavior of turkey hens and toms. *Awaiting publication* in Poultry Science.

5.1 Abstract

The impact of warm exposure during simulated transport was assessed in 12-wk-old turkey hens and 16-wk-old toms. Turkeys (96 toms, 96 hens) were randomly divided into one of four treatments: 28˚C and 20˚C temperatures with either 30% or 80% relative humidity (RH). Groups of 8 birds were crated at 83 kg/m^2 and exposed to conditions for 8h in a climate-controlled chamber before processing. Live shrink (LS), heterophil-to-lymphocyte ratio, and changes in core body temperature (CBT) and blood glucose (BG) levels were examined; meat quality was assessed by measuring initial and ultimate thigh and breast muscle pH and L*, a*, and b* colour values 27h post-mortem. Behaviour was measured using instantaneous scan sampling during the last 4h of treatment. Data were analyzed as a 2x2 factorial for temperature and humidity, significance was declared at $p \leq 0.05$. Responses of toms were also compared to responses of hens within the same treatment. Hen and tom LS was positively affected by temperature ($p<0.0001$; $p = 0.003$), from 1.5% in the 20˚C treatments to 2.8% and 2.5% in the 28˚C groups, for hens and toms, respectively. Tom ΔCBT was positively affected by temperature ($p = 0.037$), with mean increases of 0.1˚C in the 20˚C and 1.0˚C in the 28˚C groups. Ultimate breast pH was unexpectedly higher ($p = 0.023$) in toms exposed to 28˚C (pH of 5.71 at 30% and 5.67 at 80% RH) than in those exposed to 20˚C (pH of 5.71 and 5.69), but decreased with higher humidity (5.72 at 30%, 5.69 at 80% RH; $p = 0.017$). In hens, initial breast pH increased with temperature, from 6.62 at 20˚C to 6.70 at 28˚C ($p = 0.013$). Hen breast L* was higher at 80% than 30% RH, and thigh a* was negatively affected by temperature ($p = 0.047$; $p = 0.037$). Hens had larger ($p = 0.006$; $p = 0.016$) decreases in BG than toms in the 20˚C 30% and 80% RH treatments, and several differences in breast pH and a* value were noted between sexes exposed to the same conditions. LS in the 28˚C 80% RH treatment was significantly higher ($p<0.0001$) in hens (3.0%) than in toms (2.4%). The data suggest that warm-temperature exposure results in greater LS and changes in breast pH of turkeys, and CBT increase in toms. The sexes differed in response of breast pH and BG, and magnitude of LS loss, after warm exposure. Frequency of activity, panting, head-resting, and optional behaviours differed between treatments and sexes.

5.2 Introduction

Thermal stress is a major cause of mortality in transported broilers, particularly when ambient temperature exceeds 18°C, with up to 40% of DOAs attributed to this stressor (Bayliss and Hinton, 1990; Warriss et al., 2005). Despite welfare and economic concern, minimal research on the effects of thermal transport stress on another widely produced poultry species, the domestic turkey (*Meleagris gallopavo*), has been conducted. In North America, heat stress may occur year-round among transported poultry, as even during cold ambient conditions, high temperatures can develop within a trailer load (Knezacek et al., 2010). Heat stress in turkeys may result in paler meat and increased core body temperatures (Mckee and Sams, 1997; Mills et al., 1999), but fewer studies have been conducted on its effects in turkeys, compared to broilers. While broiler chicken data are used to make inferences about other domestic poultry, the species differ in metabolism, feathering, size, and age at transport, thus further research is required to determine the effects of warm temperatures on turkeys in transit.

Elevated core body temperature (CBT), or hyperthermia, can occur during both summer transport and in the centre of winter-transport loads, where poor ventilation allows for rising temperatures and humidity (Schwartzkopf-Genswein et al., 2012). CBT is approximately 40.3 to 40.8°C among turkeys housed at recommended temperatures (Mills et al., 1999; Yahav et al., 2008). While not specific to transport conditions, a study by Mills et al. (1999) found that turkey toms exposed to housing temperatures of 35°C for 4h had a CBT increase of 2.5°C, which may have implications for mortality and welfare. When examined alone, the effects of varying humidity levels on the CBT of turkeys housed at 35°C was found to be minimal (Yahav et al., 1996). Among broilers, brief exposure to high temperatures of 32.5°C - 35°C can elicit changes in CBT, as well as skin temperature, (Yahav et al., 1997; Sandercock et al., 2001; Berri et al., 2005; Lin et al., 2005). This effect is further amplified when both temperature and humidity are high (35°C and above 60% relative humidity), and may be a result of reduced effectiveness of evaporative heat loss (Sandercock et al., 2001; Lin et al., 2005). However, at exposure temperatures of 20°C to 30°C, the effects on broiler CBT are less pronounced or insignificant, and the impact of humidity is suppressed (Lin et al., 2005; Dadgar et al., 2010).

Live shrink loss has been demonstrated to increase with warmer temperatures, from 3.2% during a 12-h feed withdrawn period at 25°C, increasing to 5.7% at 34°C (Petracci et al., 2001). This increase may be attributed in part to the dehydration and increased energy demands of

panting (Whiting et al., 2007; Schwartzkopf-Genswein et al., 2012). In addition, excessive thermal panting can lead to respiratory hypocapnia as falling plasma CO_2 levels disrupt the acid-base balance with alkalosis, which contributes to heat-related mortality (Mitchell and Kettlewell, 1998; Whiting et al., 2007).

Meat quality is affected by warm ambient temperatures, with broilers and turkeys showing a decline in pH, water holding capacity, and tougher, paler meat (Babji et al., 1982; Mckee and Sams, 1997; Aksit et al., 2006), though the effect on colour is diminished in turkeys and at more moderate temperatures of 20°C - 30°C (Froning et al., 1978; Petracci et al. 2001; Bianchi et al. 2005; Bianchi et al. 2006; Dadgar et al., 2010). The observed changes in meat characteristics arise when pH drops due to the degradation of glycogen into lactic acid, and colour lightens as muscle proteins are denatured; accelerated rigor mortis may also occur (Warriss and Brown, 1987). The resulting quality issue is known as PSE – for pale, soft, and exudative meat (Sams, 1997; Owens et al., 2000; Zhang et al., 2009).

The heat-stressed broiler likely experiences greater stress than one transported in thermoneutral temperatures, as indicated by an increasing heterophil-lymphocyte ratio (HLR) with rising microclimate temperature, with heterophil numbers in particular increasing (Gross and Siegel, 1983; Mitchell and Kettlewell, 1998; Altan et al., 2003; Zhang et al., 2009). Indeed, broilers exposed to heat (39°C) for a 2-h period showed increased HLR (Warriss et al., 2005), despite the typically delayed response of this measure.

Previous broiler studies assessing heat stress have included exposure temperatures of 29°C, 34°C, and 39°C for shorter periods of time, often 1 to 2h. However, significant impacts on mortality are seen at ambient temperatures of as low as 18°C in the field (Warriss et al., 2005). The aim of this study was to assess turkey hen and tom physiology, meat quality, behaviour, and welfare when exposed to warm temperatures for an 8-h duration of simulated transport. Low and high humidity treatments were assessed to further understand the effects of these transport conditions.

5.3 Materials and Methods

All procedures and housing were approved by the University of Saskatchewan Animal Care Committee's Animal Research Ethics Board.

5.3.1 Experimental Design

A completely randomized design was used, with birds randomly sorted into 1 of the 4 exposure groups on acquisition. A total 96 hens and 96 toms were divided among four treatment conditions: 28°C with 80% relative humidity (warm-temperature, high-humidity group), 28°C with 30% relative humidity (warm-temperature, low-humidity group), 20°C with 80% relative humidity (moderate-temperature, high-humidity group), and 20°C with 30% relative humidity (moderate-temperature, low-humidity group). Each treatment group consisted of 8 same-sex birds per replicate, divided among 2 crates, which were exposed to conditions simultaneously.

A 2x2 factorial analysis, with temperature and humidity as factors, was conducted to compare the treatments with pen (8 birds) as the replicate unit. Three replications consisting of one pen each were performed. The mixed model procedure of SAS 9.4 statistics software (SAS 9.4, Cary, NC, USA) was used to analyze the data. The model was $Y = \mu + T + H + T*H + e$, where Y is the dependent variable, μ is the population mean of the variable, T is temperature (fixed), H is humidity (fixed), T*H is their interaction, and e is the random error. A two-way analysis of variance was used to make comparisons between means, and degrees of freedom were approximated with DDFM KenwardRoger. Tukey's studentized range test was used for means separation, with significance declared at $p \leq 0.05$ and trends at $p \leq 0.10$. Comparisons between sexes in the same treatment condition were made using a t-test, with the model $Y = \mu + G + e$, where G is gender (fixed). Behaviour observations were converted to percent of time budget and log-transformed for normal distribution (confirmed using the Shapiro-Wilk test) before analysis.

5.3.2 Birds and Housing

Turkeys were acquired approximately 1 wk prior to reaching typical market age, 12 and 16 wk for hens and toms, respectively, to allow them to acclimate prior to the experiment. All of the hens were obtained from one producer, and all toms from a separate single source, with each flock of 32 birds randomly divided into groups of 8 on arrival. All birds were housed at a low stocking density (6 to 12 kg/m^2) in an on-campus facility at the University of Saskatchewan. The lighting program was matched to the farm of origin with 16h light (5 lux) and 8h dark, and housing temperature ranged from 13°C to 16°C. Birds were provided *ad libitum* access to water

and complete feed obtained from the producer. Each treatment group of 8 birds was split among 2 crates prior to 8 h of exposure to the experimental conditions.

5.3.3 Environmental Chamber

The four different temperature (warm or moderate) and humidity (high or low) conditions were produced using two 2.1m by 3.4m climate-controlled chambers located on campus. Crates holding birds were moved into the chambers after the chambers were allowed to stabilize. Each of the crates (2 per chamber) was fitted with 4 USB temperature and humidity data loggers at bird level (EL USB 2+, Lascar Electronics Inc., Erie, PA, USA), and 2 additional data loggers were affixed to the front and rear walls of each chamber. A thermocouple and a humidity sensor (HM1500LF, Measurement Specialities, Inc., Impasse Jeanne Benozzi, France) were attached to each crate to monitor conditions in real-time. Temperature did not deviate from the set point by more than ±5°C, while humidity remained within 10% of the set point, excluding the first 15 minutes of the exposure period while the chamber stabilized. Lighting was sufficient to allow for behaviour monitoring, ranging from 15 to 30 lux at bird level. Air speed in both chambers was low, ranging from 0 to 0.1 m/s.

5.3.4 Data Collection

Pre-treatment Procedure and Measurements. Water was provided until capture, 2h prior to the simulated transport period, and feed was withdrawn 2h before that to result in 12h of feed withdrawal at slaughter. Each turkey was assigned an identifying number, marked with livestock paint for behaviour monitoring, wing-banded (1 band per wing), and weighed (BTDFS50-1, Berkley, Columbia, SC, USA). Average weight prior to exposure was 7.4kg for hens and 16.3kg for toms. An initial blood glucose measurement from a wing vein was taken using a needle and blood glucose meter (OneTouch UltraMini, LifeScan, Milpitas, CA, US). A miniature data logger (DS1923-F5#, Maxim Integrated, San Jose, CA) was given orally to record internal body temperature (CBT) throughout the experimental procedure, and recovered from the gizzard or crop after slaughter. CBT data during the last hour of exposure to treatment conditions were compared to baseline CBT data collected during the 1-h lairage period to determine ΔCBT.

After being prepared for the experiment, turkeys were loaded at a density of 83 kg/m^2 into sex-specific crates and transported approximately 1.5 km in a partially enclosed trailer from

the barn to the building where the chambers were located. A 1 to 1.5h lairage period was provided in a quiet, darkened room kept near barn temperature prior to moving to the test chambers.

Treatment Procedure. After lairage, birds were moved within their crates into one of the climate-controlled chambers, and exposed to one of four treatment conditions for 8 h. Video feed was monitored to observe bird condition during the experiment, and recorded for later analysis of behaviour. After the simulated transport period, birds were moved via crates to the in-building abattoir for final measurements and processing.

Post-treatment Measurements and Slaughter Procedure. A final blood glucose concentration and weight were recorded for each bird according to the same procedures followed prior to exposure. Turkeys were shackled and stunned with an electric stunning knife (VS200, Midwest Processing Systems, Minneapolis, MN, USA) at power level 5 (circa 0.16 amps, 60 Hz AC) for 30 s, or until the wing-droop response or absence of nictitating membrane reflex was noted. Stunning was followed by immediate exsanguination via the jugular vein, from which a 4-ml blood sample was collected in an EDTA (Ethylenediamine tetraacetic acid) anti-coagulation tube and placed on ice. Birds were scalded (68°C) for about 30s to 1m or until feathers were easily pulled out, plucked mechanically, and eviscerated prior to the collection of initial meat quality measurements.

Meat Quality Measurements. Directly after processing, a 5-g core sample was collected from the ventral, upper-left pectoralis major muscle and flash frozen in liquid nitrogen. Core samples were stored at -80°C until initial muscle pH could be tested using a slurry method (Stewart et al., 1984). A pH probe (Accumet, Fisher Scientific, Ottawa, ON, Canada) with a pH meter and temperature probe (Hanna H1 9025 microcomputer pH meter, North Highlands, CA, US) was used to measure breast muscle pH near the site of the core sample (Figure 5.1). Whole-bird carcasses were chilled in ice within rubber totes and refrigerated at 4°C for 24 to 27h, at which point a second core sample and pH probe measurement were taken to determine ultimate breast pH.

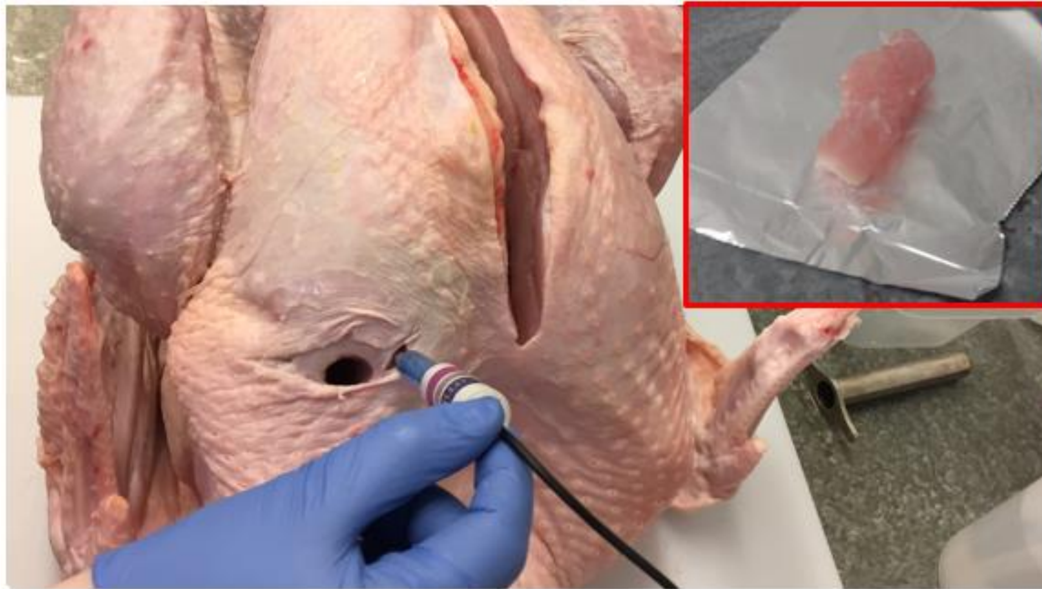


Figure 5.1: Breast pH probe measurement and core location at 27h post-mortem. Inset: breast core sample for pH testing (slurry method).

To determine meat colour, a vertical incision was made in the right breast muscle, to expose the inner portion between the major and minor pectoralis muscles. A second incision was made through the skin joining the thigh to the abdomen, to allow the femur to lay flat and expose the inner thigh muscle. After 30 minutes of exposure to air, known as blooming, colour measures were taken from the breast and thigh muscles using a Minolta colour meter (RC-400, Minolta, Ramsey, NJ, US). To account for differences in muscle fibre orientation, two readings were taken, with the device rotated 90 degrees after the first measurement. Redness, yellowness, and lightness values, a*, b*, and L*, respectively, were obtained.

To determine initial and ultimate breast pH according to the slurry method, the previously collected and flash-frozen cores were allowed to partially thaw. Five grams of breast tissue from each core was diced, placed in 20 ml of distilled water, and homogenized at 14.5k rpm for 30s (Polytron PT-3100, Kinematica, Lucerne, Switzerland) prior to pH measurement using the calibrated pH probe and microcomputer.

Blood Smear Preparation and Staining. Blood smears were prepared according to the 2 slide wedge method, wherein a small drop of blood was transferred from the sample tube via a capillary tube 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/lymphocyte ratios (HLR) were

determined by viewing the slides under an oil immersion lens (1000x magnification), as seen in Figure 5.2. Leukocytes were identified and counted until a total of 100 combined heterophils and lymphocytes was reached, and the number of the former was divided by the latter to produce the HLR. The mean of three HLR ratios was calculated for each slide.

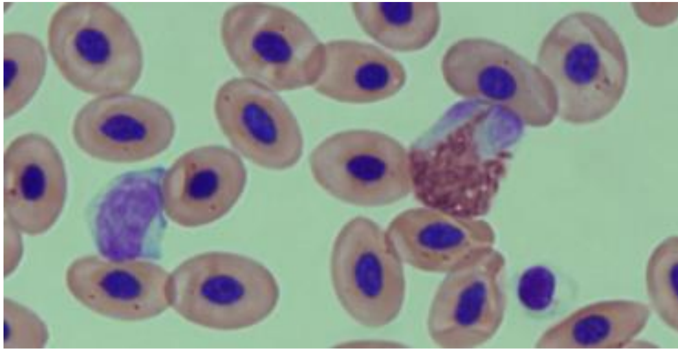


Figure 5.2: A lymphocyte (left) and heterophil (right) in the domestic turkey.

5.3.5 Behavioural Analysis

Behaviour data were collected from the last 4h of recorded video using instantaneous scan sampling at 5-minute intervals, with up to 5s prior and after the sampling point observed to determine which behaviour was occurring at that time (Lehner, 1998). The behaviours of all 4 birds within each crate were recorded by 2 observers, and the resulting data averaged to produce a time budget for each pen (replicate unit). Data validation was performed between the 2 observers via a paired comparison of one replicate per treatment in SAS 9.4. Proc TTest was used to compare datasets, which did not differ significantly. Recorded behaviours, partially adapted from Webster (2000), are defined in Table 5.1, with additional categories created for combinations of positions and behaviours as needed.

Table 5.1: All of the different behaviours observed during the study and their defining criteria. Behaviour categories were divided based on position (standing or sitting), and all were mutually exclusive with the exception of panting (Webster, 2000).

Still	The bird is motionless
Active	The bird is standing or attempting to rise, and moving feet or wings, changing position, or changing location in the crate
Sit at Rest	Bird is in the sitting position, motionless with its body contacting the floor of the crate
Survey	Quick head movements in an alert bird, suggesting visual surveillance of the environment
Peck	The beak is used to peck at other birds or objects, including sensors and the floor of the crate
Huddle	Birds are grouped closely in one area of the crate, with minimal movement OR birds are actively attempting to 'burrow' beneath or between other birds
Preen	The beak is used to comb through or manipulate any area of feathers on the bird's own body
Pant	The bird breathes through an open beak (not mutually exclusive)
Head Rest	The head is rested heavily on the floor or slats of the crate, or another bird, panting may occur but the bird does not otherwise stand or move
Skin Expose	The unfeathered skin of the neck is exposed, feathers are spread to expose bare skin of the torso, or wings are drooped.

5.4 Results and Discussion

5.4.1 Physiological Measures

Physiological data for hens are presented in Table 5.2, and tom data are in Table 5.3. Live shrink was significantly higher with increasing temperature among both hens and toms ($p<0.0001$; $p = 0.003$), with a tendency for a positive effect of RH on hens ($p = 0.090$). For the hens and toms exposed to moderate temperatures, live shrink amounted to 1.46% and 1.44%, respectively, increasing to 2.77% and 2.50% in the warm-exposed birds. This is indicative of increased energy demand on birds in warmer conditions, exacerbated by dehydration and the reduced efficiency of panting in high-humidity environments (Dawson and Whittow, 1994; Yahav et al., 1995; Yahav et al., 1997).

Table 5.2: Physiological and meat quality measures taken in turkey hens exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH, and the p-values for significant differences and trends.

Measure	Treatment				P-value			
	20°C 30% RH	20°C 80% RH	28°C 30% RH	28°C 80% RH	Temp	RH	Temp* RH	SEM
Live Shrink (%)	1.45a	1.46a	2.54b	3.00b	<0.0001	0.090	NS	0.211
ΔGlucose (mmol/L)	-2.29	-1.65	-1.68	-1.90	NS	NS	NS	0.179
ΔCBT (°C)	0.31	-0.08	-0.01	0.31	NS	NS	NS	0.123
HLR	1.54	1.60	1.74	1.57	NS	NS	NS	0.09
Initial Breast pH (slurry)	6.54	6.59	6.52	6.56	NS	0.091	NS	0.031
Initial Breast pH (probe)	6.61a	6.63a	6.73b	6.66b	0.013	NS	0.066	0.022
Ultimate Breast pH (slurry)	5.66	5.63	5.64	5.65	NS	NS	NS	0.012
Ultimate Breast pH (probe)	5.71	5.67	5.71	5.69	NS	NS	NS	0.017
Ultimate Thigh pH (probe)	5.99	5.88	5.93	5.91	NS	NS	NS	0.035
Thigh L*	49.87	50.69	51.27	51.02	NS	NS	NS	0.311
Thigh a*	14.61a	13.96a	13.13b	13.49b	0.037	NS	NS	0.232
Thigh b*	0.49	0.32	0.90	0.88	NS	NS	NS	0.135
Breast L*	50.56a	52.54b	51.08a	51.29b	NS	0.047	0.092	0.280
Breast a*	5.02	4.75	4.89	4.80	NS	NS	NS	0.084
Breast b*	-3.53	-3.09	-2.93	-2.96	NS	NS	NS	0.109

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens (8 birds/replication), except CBT for the 20°C 30% RH treatment (average response of 16 hens).
[CBT] Core Body Temperature
[HLR] Heterophil Lymphocyte Ratio

Table 5.3: Physiological and meat quality measures taken in turkey toms exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH, and the p-values for significant differences and trends.

	Treatment				P-value			
Measure	20°C 30% RH	20°C 80% RH	28°C 30% RH	28°C 80% RH	Temp	RH	Temp *RH	SEM
Live Shrink (%)	1.61ab	1.47a	2.62b	2.38ab	0.003	NS	NS	0.176
ΔGlucose (mmol/L)	-0.59	-1.32	-0.84	0.16	NS	NS	NS	0.305
ΔCBT (°C)	-0.27a	0.09a	0.44b	1.64b	0.037	NS	NS	0.284
HLR	1.82	1.33	1.51	2.52	NS	NS	0.089	0.207
Initial Breast pH (slurry)	6.45	6.43	6.48	6.46	NS	NS	NS	0.029
Initial Breast pH (probe)	6.73	6.76	6.77	6.73	NS	NS	NS	0.022
Ultimate Breast pH (slurry)	5.70ab	5.71ab	5.73a	5.67b	NS	0.073	0.017	0.014
Ultimate Breast pH (probe)	5.71a	5.69a	5.71b	5.67b	0.023	NS	0.098	0.008
Ultimate Thigh pH (probe)	5.81	5.87	5.84	5.88	NS	NS	NS	0.018
Thigh L*	50.20	49.70	49.32	50.46	NS	NS	NS	0.309
Thigh a*	12.80	13.89	11.39	12.47	NS	NS	NS	0.896
Thigh b*	-0.41	0.46	-0.73	0.27	NS	NS	NS	0.444
Breast L*	50.39	50.01	50.05	50.70	NS	NS	NS	0.343
Breast a*	6.40	5.52	7.40	6.33	NS	NS	NS	0.531
Breast b*	-2.20	-2.58	-2.00	-2.26	NS	NS	NS	0.199

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05). Means represent the average response of 24 toms (8 birds/replication).
[CBT] Core Body Temperature
[HLR] Heterophil Lymphocyte Ratio

While a live shrink of approximately 2.8% in hens and 2.5% in toms is within normal expectations for broiler and turkey transport, it is nearly double what was observed in the 20°C treatments and represents an economic loss that may be avoided (Duke et al., 1997; Petracci et al, 2001; Northcutt et al., 2003; Dadgar et al., 2011). The differences between the recorded data herein and those presented in published literature were likely, at least partially, the result of the initial weight being recorded 2 hours after feed withdrawal began. Live shrink has not been related directly to welfare, but at the more extreme high temperatures experienced during routine transport, it may serve as an indicator of metabolic stress. No differences in Δ blood glucose (mmol/L) were detected between treatment conditions for hens nor tom.

Among toms, there was a significant effect ($p = 0.037$) of exposure temperature on ΔCBT (°C), with males in the 20°C treatments experiencing a decrease of 0.09°C, and those in the 28°C treatment an increase of 1.04°C. The rise in CBT observed among toms is sufficient to indicate that hyperthermia was occurring, implying that the thermoregulative abilities of the male turkeys had been exceeded (Yahav et al., 1995). No differences in ΔCBT were detected among hens. Hyperthermia in broilers has been linked to a significant portion of transport-related mortality, along with the panting and disruption to acid-base balance that follows it, and suggests that welfare has been compromised (Mitchell and Kettlewell, 1998; Whiting et al., 2007). This is supported by the tendency ($p = 0.089$) for an increased HLR in toms, positively influenced by the interaction of temperature and humidity. In particular, toms in the 28°C 80% RH treatment had a HLR of 2.5, suggesting an increase in stress and concomitant detriment to welfare in these conditions (Gross and Siegel, 1983; Mitchel and Kettlewell, 1998). No differences in HLR were detected among hens.

When the physiological responses of male and female turkeys were compared (Table 5.4), a few differences were noted in the way they responded to each of the treatments. Delta blood glucose (mmol/L) varied significantly between hens and toms in the 20°C 30% RH and 20°C 80% RH treatments ($p = 0.006$; $p = 0.016$); with toms experiencing smaller decreases in blood glucose level (0.59 mmol/L and 1.32 mmol/L) than hens (2.29 mmol/L and 1.65 mmol/L) over the exposure period. In addition to any inherent sex differences in blood glucose response, the smaller size and younger age of the hens likely impacted this measure. While the effects of feed withdrawal certainly play a role in its decrease in both sexes (Nijdam et al., 2005), it is possible that the smaller hens had less available energy to draw on in coping with simulated

transport, and thus experienced a larger drop in blood glucose. The change in this measure was not significantly different between hens and toms at warmer temperatures, though numerically hens still experienced a greater decrease.

Table 5.4: All physiological measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Live Shrink (%)				
20°C 30% RH	1.45	1.61	NS	0.134
20°C 80% RH	1.46	1.47	NS	0.162
28°C 30% RH	2.54	2.62	NS	0.102
28°C 80% RH	3.00a	2.38b	<0.0001	0.140
ΔGlucose (mmol/L)				
20°C 30% RH	-2.29a	-0.59b	0.006	0.688
20°C 80% RH	-1.65a	-1.32b	0.016	0.740
28°C 30% RH	-1.61	-0.64	NS	0.369
28°C 80% RH	-1.95	-0.38	NS	0.559
ΔCBT (°C)				
20°C 30% RH	0.35[1]	-0.27	NS	0.299
20°C 80% RH	-0.08	0.09	NS	0.100
28°C 30% RH	0.01	0.35	NS	0.176
28°C 80% RH	-0.003	1.32	0.097	0.405
HLR				
20°C 30% RH	1.54	1.82	NS	0.129
20°C 80% RH	1.60	1.33	NS	0.119
28°C 30% RH	1.66	1.46	NS	0.165
28°C 80% RH	1.33	2.22	NS	0.341

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens or toms (8 birds/replication), except CBT for the 20°C 30% RH treatment (average response of 16 hens, 24 toms).
[CBT] Core Body Temperature
[HLR] Heterophil Lymphocyte Ratio

Live shrink was significantly higher in hens than in toms exposed to the 28°C 80% RH (p = <0.0001), with a mean live shrink of 3.00% and 2.38%, respectively, suggesting that relatively more energy was expended by hens in coping with these conditions. This lends support to the notion that hens have less available energy to endure transport, and must dip relatively further than toms into their bodily reserves to cope with the same conditions. However, live shrink did not differ between sexes in other treatment conditions.

While hens may experience some greater energy demands, toms in the 28°C 80% RH group tended (p = 0.097) to experience a larger change in CBT - with a mean increase of 1.32°C in males and mean decrease of 0.003°C in females. This suggests the male turkeys are less able to cope with an environment above thermoneutral. The larger size of toms, while potentially beneficial in terms of live shrink and blood glucose, likely hinders their ability to thermoregulate as successfully as it provides a relatively lower surface area from which to lose heat in warm conditions (MacLeod and Hocking, 1993; Dawson and Whittow, 1994).

5.4.2 Meat Quality Indicators

Meat quality is of importance not only to processor and consumer, but also serves a window into further biological changes experienced by the live turkey in the face of heat stress. Measures such as pH and colour can serve as indicators of other more direct measures of quality, as well as provide insight into the metabolic activity which is occurring. Several changes in meat quality were observed for both hens and toms, mainly affected by temperature, but with some impact of humidity as well.

In hens (Table 5.2), initial breast pH (probe method) was significantly (p = 0.013) higher in the warm exposure groups (pH = 6.70) than in those exposed to moderate temperatures (pH = 6.62). This measure also had a tendency to be positively affected (p = 0.066) by the temperature-humidity interaction, while initial breast pH according to the slurry method had a tendency (p = 0.0907) to be positively affected by RH. This difference in initial muscle pH was unexpected, as in past research exposure to warm temperatures has resulted in a lower initial (and ultimate) pH (Sandercock et al., 2001; Askit et al., 2003). The effects on muscle pH were, however, transient, and there were no impacts of increasing temperature or humidity on the ultimate breast or thigh pH of hens, indicating that meat quality was not overly compromised by these conditions. Despite the unexpected changes in pH, colour changes which occurred were in line with previous broiler and turkey research (Froning et al., 1978; Bianchi et al., 2006), particularly increased breast lightness (L*), used as an indicator of low-pH, PSE meat. This measure was positively affected by RH (p = 0.047), with a mean L* of 50.82 in the 30% RH and 51.77 in the 80% RH groups. The L* value also had a tendency (p = 0.092) to be positively affected by the temperature-humidity interaction. Thigh redness (a*) decreased with increasing temperature, from a mean of 14.29 in the 20°C treatments to 13.31 in the 28°C treatments (p = 0.037), though

the role of this colour value in final meat quality is less clear (Bianchi et al. 2006; Dadgar et al., 2010). While lightness on its own is not sufficient to indicate that quality is compromised, consumer acceptance may be affected if lighter cuts are packaged with darker ones, and the change may represent a mild or early version of the PSE defect, wherein meat is pale, soft, and exudative, and may be condemned at the processor (Owens et al., 2000).

Table 5.5: All initial and ultimate pH measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Initial Breast pH (slurry)				
20°C 30% RH	6.52	6.45	NS	0.048
20°C 80% RH	6.56	6.76	NS	0.044
28°C 30% RH	6.55	6.45	NS	0.060
28°C 80% RH	6.66a	6.47b	0.048	0.053
Initial Breast pH (probe)				
20°C 30% RH	6.73	6.73[1]	NS	0.058
20°C 80% RH	6.66	6.76	NS	0.036
28°C 30% RH	6.66a	6.78b	0.016	0.029
28°C 80% RH	6.68	6.73	NS	0.025
Ultimate Breast pH (slurry)				
20°C 30% RH	5.64	5.70	NS	0.022
20°C 80% RH	5.65a	5.71b	0.031	0.017
28°C 30% RH	5.65	5.72	NS	0.030
28°C 80% RH	5.63	5.69	NS	0.022
Ultimate Breast pH (probe)				
20°C 30% RH	5.71	5.71[1]	NS	0.002
20°C 80% RH	5.69	5.69	NS	0.020
28°C 30% RH	5.70	5.72	NS	0.021
28°C 80% RH	5.70	5.75	NS	0.023
Ultimate Thigh pH (probe)				
20°C 30% RH	5.93	5.83[1]	NS	0.036
20°C 80% RH	5.91	5.87	NS	0.043
28°C 30% RH	5.98	5.85	NS	0.044
28°C 80% RH	5.93	5.91	NS	0.035

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens or toms (8 birds/replication), except initial breast pH (probe), ultimate breast pH (probe), and ultimate thigh pH (probe) for the 20°C 30% RH treatment, which includes the average response of 16 toms and 24 hens.

Table 5.6: All meat colour measures taken in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH and the p-values for significant differences and trends.

Measure	Hens	Toms	P-Value	SEM
Thigh L*				
20°C 30% RH	51.27	50.20	NS	0.482
20°C 80% RH	51.02	49.70	NS	0.524
28°C 30% RH	50.15	49.38	NS	0.361
28°C 80% RH	50.47	50.48	NS	0.459
Thigh a*				
20°C 30% RH	13.13	12.80	NS	0.566
20°C 80% RH	13.49	13.89	NS	0.556
28°C 30% RH	14.63	10.61	NS	1.501
28°C 80% RH	14.00	13.38	NS	0.974
Thigh b*				
20°C 30% RH	0.90	-0.41	NS	0.406
20°C 80% RH	0.88	0.46	NS	0.478
28°C 30% RH	0.56	-0.83	NS	0.546
28°C 80% RH	0.26	0.50	NS	0.556
Breast L*				
20°C 30% RH	51.08	50.39	NS	0.334
20°C 80% RH	51.29	50.01	NS	0.479
28°C 30% RH	50.79	50.05	NS	0.558
28°C 80% RH	52.50	50.70	NS	0.560
Breast a*				
20°C 30% RH	4.89	6.40	NS	0.624
20°C 80% RH	4.80a	5.52b	0.045	0.195
28°C 30% RH	4.91	8.03	0.093	0.946
28°C 80% RH	4.68	6.11	NS	0.570
Breast b*				
20°C 30% RH	-2.93	-2.20	NS	0.242
20°C 80% RH	-2.96	-2.58	NS	0.131
28°C 30% RH	-3.41	-1.90	NS	0.467
28°C 80% RH	-3.06	-2.46	NS	0.224

[a, b] Means with common letters within a main effect do not differ significantly (P<0.05).
[1] Means represent the average response of 24 hens or toms (8 birds/replication)

In turkey toms (Table 5.3), no effects of treatment were seen on the initial breast pH, despite there being several differences in ultimate breast pH. According to the slurry method, ultimate breast pH was negatively affected by the temperature-humidity interaction (p = 0.017), with the lowest pH of 5.67 observed in the 28°C 80% RH treatment, the highest (5.73) in the

28°C 30% RH treatment, and the 20°C 30% RH and 20°C 80% RH treatment producing mean ultimate breast pH values of 5.70 and 5.71, respectively. This measure also had a tendency ($p = 0.073$) to be negatively affected by RH. Ultimate breast pH according to the probe method also decreased with increasing temperature, with a mean pH of 5.70 in the 20°C treatments and 5.69 in the 28°C treatments, with a tendency ($p = 0.098$) for a negative effect of the temperature-humidity interaction. No differences in tom ultimate thigh pH were detected.

While initial muscle pH changes may serve as an early sign that quality changes have occurred, ultimate pH is of greater concern (Fernandez et al., 2002), as it represents the final quality of the meat, a change in what consumers will receive. The decrease in breast muscle pH of toms was in line with expectations for warm exposure based on past research (Sandercock et al., 2001; Askit et al., 2003), and persisted into final measurements, unlike the pH changes in hens. However, the lowered pH was not so severe as to qualify as PSE, though it may be beginning to occur (Sams and McKee, 1997), and considered alongside the total lack of change in meat colour, it is unlikely that meat quality was compromised among toms in these conditions.

When comparing the responses of hen and tom meat quality to each of the different treatments, a few significant differences were detected (Table 5.5). Hens had a lower initial breast pH (probe method) than toms in the 28°C 30% RH, 6.66 and 6.78, respectively ($p = 0.016$), and a lower ultimate breast pH (slurry method) in the 20°C 80% RH treatment, with a mean pH of 5.65, compared to 5.71 among toms ($p = 0.031$). This was somewhat unexpected, as the physiological data suggested toms were more negatively affected by warm exposure. This notion was upheld in the 28°C 80% RH treatment, where initial breast pH (slurry method) was higher in hens than toms, with means of 6.66 and 6.47 respectively ($p = 0.048$). Numerically, similar (but insignificant) differences in mean ultimate breast pH of hens and toms were apparent in all treatments according to the slurry method, with hen pH ranging from 5.63 to 5.65 and tom pH from 5.69 to 5.72. This suggests that the difference observed in this measure may be dependant on differences between the hens and toms (such as age or size), and is less likely influenced by differing responses to warm temperature or high humidity. Similar patterns are observed in the ultimate breast pH according the probe method, and ultimate thigh pH, though no significant differences were detected.

In terms of meat colour values (Table 5.6), only one measure differed significantly between sexes, breast redness (a*). This measure was significantly lower in hens than toms in the 20°C 80% RH treatment, 4.80 and 5.52, respectively (p= 0.045), and tended (p = 0.093) to be lower in hens in the 28°C 30% RH treatment, with mean values of 4.91 and 8.03, respectively. Breast meat redness was also numerically (but not significantly) decreased in hens in the remaining two treatments. This again suggests a sex-inherent variation in breast a*, potentially due to the increased age or size of the toms, rather than a difference in response to the exposure conditions.

5.4.3 Behavioural Analysis

Several differences in time budgets between treatment conditions were apparent, influenced by both temperature and humidity as well as their interaction (Table 5.7). Among hens, time spent merely sitting still or at rest decreased (p = 0.047; p = 0.031) with increasing temperature and humidity, from between 82.1% to 86.5% in both moderate-temperature treatments and the low humidity, warm-temperature treatment, down to 49.5% of the time budget in the 28°C 80% RH group. Time spent on the optional behaviour of pecking (at the environment or other birds) was also decreased in both warm-temperature treatments (p = 0.028), from a mean of 2% in the 20°C treatments to 1.1% in the 28°C treatments. Instead, more time was spent on panting, as expected when coping with temperatures exceeding thermoneutral (Dawson and Whittow, 1994). Panting behaviour occurred significantly more often with increasing temperature, humidity, and their interaction (p = <0.0001; p = 0.001; p = 0.013), from less than 1% of the time budget in both 20°C treatments, to 4.4% in the 28°C 30% RH treatment, and 31.4% in the 28°C 80% RH hens. Hens also tended to head-rest more often with increasing temperature (p = 0.071), a behaviour where the head is rested on the crate or on companions. This behaviour may be an alternative position of resting that allows the unfeathered skin of the neck to be exposed, which is thought to aid in thermoregulation among turkeys (Buchholz, 1996). It is possible fatigue plays some role, but the continuation of panting suggests this behaviour is not performed due to complete exhaustion.

Table 5.7: Grouped behaviour data (%) in turkey hens and toms exposed to 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, and 28°C 80% RH and the p-values and standard error. Measures are mutually exclusive.

	Behaviour	20°C 30%RH	20°C 80%RH	28°C 30%RH	28°C 80%RH	Temp	RH	Temp *RH	SEM
Hens	Active	5.1	7.2	4.7	5.1	NS	NS	NS	0.51
	Stand Still	2.3	6.5	4.0	4.2	NS	NS	NS	0.67
	Sit Still/Rest	86.5a	82.1a	83.8a	49.5b	0.036	0.027	0.068	5.17
	Preen	1.3	1.3	0.6	0.6	NS	NS	NS	0.26
	Survey	1.0	0.4	0.3	0.3	NS	NS	NS	0.16
	Peck	2.0a	1.9a	0.9b	1.2b	0.028	NS	NS	0.20
	Head Rest	0a	0.1a	0.7a	5.1b	0.071	NS	NS	0.82
	Pant	0.1c	0.7c	4.4b	31.4a	<.0001	0.001	0.013	3.70
	No Obsv	0	0.3	0.3	2.8	NS	NS	NS	0.69
Toms	Active	4.3	2.4	2.9	3.1	NS	NS	NS	0.37
	Stand Still	0.8	0.6	0.6	0.1	NS	NS	NS	0.14
	Sit Still/Rest	58.7	33.2	21.0	6.9	NS	NS	NS	7.67
	Preen	0.4	0.3	0	0.1	0.054	NS	NS	0.09
	Survey	1.4	1.4	1.4	1.0	NS	NS	NS	0.37
	Peck	0.6	0.4	0.3	0.1	0.088	NS	NS	0.10
	Head Rest	3.8b	33.0a	24.0b	27.0a	NS	0.028	NS	5.54
	Pant	15.9b	33.8b	49.0a	60.1a	0.039	NS	NS	8.58
	Expose Skin	0b	0b	0b	12.4a	0.003	0.002	0.003	2.20
	No Obsv	13.9	7.9	20.3	15.5	NS	NS	NS	3.71

Toms displayed several similar alterations of time budget, tending to spend less time on the optional behaviours of preening and pecking (p = 0.054; p = 0.088) with increasing temperature, and significantly greater time on behaviours considered relevant to thermoregulation. Panting occurred more frequently as temperature increased, as expected, from

a mean of 24.9% in the 20°C treatments up to 54.6% in the 28°C treatments (p = 0.039), while head-resting occurred more often with higher humidity, with a mean of 13.9% in the 30% RH groups up to 30% of the time budget in the 80% RH group (p = 0.028). This supports the role of head-resting as a thermoregulatory behaviour, and one which can be effectively utilized with increasing humidity - unlike panting, which has reduced efficiency as it relies on evaporative heat loss (Yahav et al., 1995; Lin et al., 2005). Toms in the 28°C 80% RH treatment also displayed skin-exposing behaviour with a frequency of 12.4% of their time budgets, where wings were spread away from the body to expose the bare areas of the torso, and feathers lowered to expose the bare skin of the neck. The frequency of this measure was positively affected by temperature, humidity, and the temperature-humidity interaction (p = 0.003; p = 0.002; p = 0.003). This behaviour further aids in thermoregulation, as heat is more readily lost from bare skin than from beneath the insulation of feathers (Dawson and Whittow, 1994). It is not clear why this behaviour was performed only by toms, and only in the warmest, most humid treatment, but it may be related to the space limitations of the crate – this behaviour often required the bird be standing, and at some distance from its companions, so it may have only been performed in conditions where other methods of thermoregulation were inadequate.

In comparing the behavioural responses of hens and toms within a treatment, several differences in time budgets were noted. In the moderate-temperature, low-humidity treatment (Table 5.8), toms had a tendency (p = 0.072) to spend less time standing still than hens, 0.8% and 2.3% respectively, and spent significantly less time huddling (0% and 1.4%; p = 0.012) or pecking (0.6% and 2%; p = 0.011). Head-resting behaviour comprised 3.8% of tom time budgets, and was not seen in hens (p = 0.039), and panting, also not observed in hens, comprised 15.9% of tom time budgets (p = 0.006). It is likely that even at this exposure temperature, toms were experiencing a non-thermoneutral environment, and were coping with the use of thermoregulatory behaviours. Similar effects were seen in the 20°C 80% RH or moderate-temperature, high-humidity treatment (Table 5.8), with toms again spending more time head-resting than hens, 33% and 0.1% of respective time budgets (p = 0.002), as well as a numerically greater (but statistically insignificant) amount of time spent panting. Hens spent more time (p = 0.012) standing still than toms, 5.8% and 0.6% of time budgets, respectively, and also spent more time active (7.2% and 2.4%; p = 0.015). Hens also spent more time engaged in optional pecking behaviour, pecking for 1.9% of observed time compared to 0.4% in toms (p = 0.024).

Table 5.8: Grouped behaviour data (%) in turkey hens and toms exposed to 20°C 30% RH and 20°C 80% RH with p-values and standard errors. Measures are mutually exclusive, except panting.

	Behaviour	Hens	Toms	P	SEM
20°C 30% RH	Active	5.1	4.3	NS	0.54
	Stand Still	2.3	0.8	0.072	0.42
	Sit at Rest	86.5	58.7	NS	8.76
	Huddle	1.4a	0b	0.012	0.36
	Preen	1.3	0.4	NS	0.44
	Survey	1.0	1.4	NS	0.54
	Peck	2.0a	0.6b	0.011	0.34
	Head Rest	0b	3.8a	0.039	1.10
	Pant	0b	15.9a	0.006	4.77
	No Obsv	0	13.9	NS	4.86
20°C 80% RH	Active	7.2a	2.4b	0.015	1.24
	Stand Still	5.8a	0.6b	0.012	1.56
	Sit at Rest	82.1	33.2	NS	13.46
	Preen	1.3	0.3	NS	0.37
	Survey	0.4	1.4	NS	0.31
	Peck	1.9a	0.4b	0.024	0.39
	Head Rest	0.1b	33.0a	0.002	9.13
	Pant	0.7	33.8	NS	15.60
	No Obsv	0.3	7.9	NS	3.03

Table 5.9: Grouped behaviour data (%) in turkey hens and toms exposed to 28°C 30% RH and 28°C 80% RH each treatment with p-values and standard errors. Measures are mutually exclusive, except panting.

	Behaviour	Hens	Toms	P	SEM
28°C 30% RH	Active	4.7	2.9	NS	0.72
	Stand Still	4.0a	0.6b	0.011	0.92
	Sit at Rest	83.8a	21.0b	0.018	14.56
	Preen	0.6a	0b	0.020	0.15
	Survey	0.3	1.4	NS	0.57
	Peck	0.9	0.3	0.070	0.22
	Head Rest	0.7b	24.0a	0.022	8.24
	Pant	4.4b	49.0a	0.003	10.59
	No Obsv	0.3	20.3	NS	6.41
28°C 80% RH	Active	5.1	3.1	NS	0.62
	Stand Still	4.2a	0.1b	0.001	0.94
	Sit at Rest	49.5a	6.9b	0.010	10.65
	Preen	0.6	0.1	NS	0.18
	Survey	0.3	1.0	NS	0.31
	Peck	1.2a	0.1b	0.032	0.30
	Head Rest	5.1	27.0	0.071	6.35
	Pant	31.4	60.1	0.071	7.63
	Expose Skin	0b	12.4a	0.011	4.18
	No Obsv	2.8	15.5	NS	5.42

These patterns continued in the warm-temperature, low-humidity treatment (Table 5.9). Hens spent more time ($p = 0.011$) standing still than toms, 4% and 0.6% of time budgets, respectively, and more time sitting at rest (83.8% and 21%; $p = 0.018$). Hens also engaged in more frequent preening behaviour (0.6% and 0%; $p = 0.020$), and tended to peck more often ($p = 0.070$), though these were uncommon behaviours in both sexes. Toms panted more frequently ($p = 0.003$) than hens in these conditions, 49% and 4.4% of respective time budgets, and also

performed more head-resting behaviour (24% and 0.7%; p = 0.003), indicate of their greater thermoregulatory needs.

This disparity in response of hens and toms to warm conditions became less apparent in the 28°C 80% RH treatment, where hens only had a tendency to pant and head-rest less than toms (p = 0.071; p = 0.071). However, hens still spent more time standing still than toms, 4.2% and 0.1%, respectively (p = 0.001), and more time sitting at rest (49.5% and 6.9%; p = 0.010). They also pecked more frequently (1.2% and 0.1%; p = 0.032), though again this was a relatively uncommon behaviour. Toms spent more time with skin exposed, 12.4% of observed time budget, a behaviour hens did not perform at all (p = 0.011). Overall, the relatively consistent behavioural differences in hens and toms across treatments demonstrates that toms were more heat-stressed than hens, and were compelled to spend much more of their time on thermoregulation at the expense of resting or optional behaviours.

5.5 Conclusions

Physiology and meat quality measures were affected by simulated warm transport among both turkey hens and toms, though heat stress was not severe enough to detriment meat quality. Hens, which displayed increased live shrink and a drop in blood glucose, experienced a metabolic demand which exceeded their reserves, as the demands of panting exacerbated the normal effects of feed withdrawal. The consistently larger drop in blood glucose of hens when compared to toms lends support to the notion that the smaller size of hens may be impacting their relative energy reserves. Toms, on the other hand, had increased live shrink, an increase in ΔCBT, and a tendency for a higher HLR, suggesting that heat stress was occurring, and their ability to cope with this stressor was beginning to be exceeded – temperature homeostasis could no longer be maintained, and hyperthermia was occurring. In combination with behaviour data, which indicated toms were engaged in thermoregulatory behaviour across treatments and with greater frequency than hens, it is likely that the larger size and advanced age of toms have a strong impact on their ability to cope with heat stress. The reduced surface area relative to body weight inherent to larger birds decreases the area from which heat can be lost, though additional sex differences may have influenced hen and tom responses to warm conditions.

Meat quality was not compromised among hens, as changes in pH did not persist beyond chilling when the ultimate pH measurements were made. Additionally, the changes noted in

initial pH were not in line with expectations for the effects of warm exposure, suggesting hens were able to cope with the thermal stressor. Further exploration into the effects of mild and severe heat stress in turkeys may be beneficial in determining the biochemical changes which occur in muscle tissue, and how these changes differ among broilers, female turkeys, and male turkeys. Despite the unintuitive and minor changes in muscle pH of hens, a few colour differences were present in the breast muscle, the most relevant being the increased L* value in warm-exposed hens. While lightness has been used to predict low pH in broiler and turkey meat, as well as the PSE defect, the changes observed in this study were not sufficient to have a major effect on consumer acceptance, and are not considered likely to be detrimental to meat quality.

Though meat quality in toms was not dramatically affected by warm transport, with no apparent changes in colour or initial pH, there were differences which persisted into the final meat quality in terms of ultimate breast pH. Unlike in hens, muscle pH was decreased in toms after warm exposure, in line with expectations based on past heat stress research. While ultimate pH is of greater interest in terms of quality, as it affects the product consumers will receive, the changes were not extreme enough to suggest the PSE defect was occurring. While the pH changes warrant consideration from a processing point of view, as well as a potential indicator of early PSE-type changes, it is unlikely that overall meat quality was severely compromised in toms in these conditions.

Neither sex showed a particularly strong response of HLR to warm exposure. Heat stress has been shown to impact HLR in past research, even with a shorter duration of exposure than was used in this study. However, the lack of baseline, pre-transport data to compare with, and the high level of variation between individual birds, may have obscured the effects of warm conditions on this measure. Despite the lack of significant effects, the HLR values were relatively high, and considered in combination with physiological and behavioural data it is probable that birds were stressed and welfare was compromised. The behaviour of transported turkeys of both sexes was disrupted by warm conditions, with toms displaying particularly strong signs of discomfort and stress. Turkeys were also likely exposed to hunger and thirst beyond that of thermoneutral transport, as a result of the dehydrating effects of thermoregulating in warm conditions and the metabolic stress imposed. Toms likely experienced greater detriment to welfare than hens, though neither sex demonstrated clear signs of severe distress.

Despite the impact on welfare, the selection of 28°C for the warm temperature treatments was insufficient to provoke severe heat stress or consistent changes in meat quality. Instead, 30°C may be a more appropriate temperature for future research, while preliminary trials suggest 35°C is likely too hot, and has the potential for mortality and poor welfare. Nevertheless, some effects on meat quality and physiology were still apparent, and the doubling of live shrink loss suggests that exposure to these conditions has major effects on productivity. Transport at these temperatures is unlikely to detriment meat quality, particularly in hens, which appeared to cope more effectively with warm and humid conditions than toms. Further research at warmer temperatures and comparison of hen and tom field data would be useful to determine acceptable transport thresholds and the point at which thermal stress becomes excessive.

6.0 DISCUSSION AND CONCLUSIONS

There is currently a deficit in research on the effects of transport in adverse conditions on turkeys, despite the fact they are transported year-round in highly variable temperatures. In this study, the effects of warm and cold exposure on selected behavioural and physiological indicators of welfare, metabolism, and meat quality were assessed to further understand turkeys' thermoregulatory coping abilities. The selected exposure conditions were not extreme enough to induce consistent and widespread physiological changes, though the data suggest moderate thermal stress was induced among toms in the 28°C treatments, and hens in -18°C temperature condition. Changes in core body temperatures in these conditions indicate that birds were beginning to reach the limits of their ability to cope with the temperatures to which they were exposed for the 8-hour duration. Live shrink was also significantly increased in both hens and toms exposed to 28°C and -18°C, indicating additional energy expenditure was necessary during thermally adverse transport, as well as representing a source of economic loss for the industry.

The behavioural analyses showed that in both -18°C and 28°C exposure groups, and at times in the moderate 20°C groups, thermoregulatory behaviours were employed. While a moderate temperature of 20°C was chosen to serve as a control for comparisons, a clearer representation of the effects of cold and warm transport would have been gained by using a thermoneutral control equal to housing temperature. In the turkeys we obtained for this study, this temperature would have been near 15°C (barn temperature). Thermoregulatory behaviour in the 28°C and 20°C treatments consisted mainly of panting, indicating thermoneutral temperatures had been exceeded, and behavioural strategies were needed to mitigate the effects of heat stress. The additional energy required to pant likely explains a portion of the increase in live shrink, though in no condition did live shrink exceed the expected range (2 to 5%) for turkeys subjected to 12-hour feed withdrawal (Duke et al., 1997). As higher humidity decreased the effectiveness of panting, it occurred with greater frequency, and additional heat-coping methods (head-resting and skin exposure) were employed. The impacts on physiology were more pronounced in the 28°C exposure groups, particularly in the toms exposed to 28°C with high humidity, who may be nearing the upper limit of their ability to successfully cope. This is apparent in the patterns of CBT variation throughout the exposure period, shown in Figure 6.1. The ΔCBT of toms (using each individual bird's mean CBT during the last 30 minutes of lairage

as a baseline) in the 28°C treatment continuously increased, showing clear signs of hyperthermia and an inability to maintain thermal homeostasis in these birds. This pattern was not evident in other treatments, where ΔCBT remained relatively flat over the exposure period, though it is important to note statistical analysis was not performed on these data.

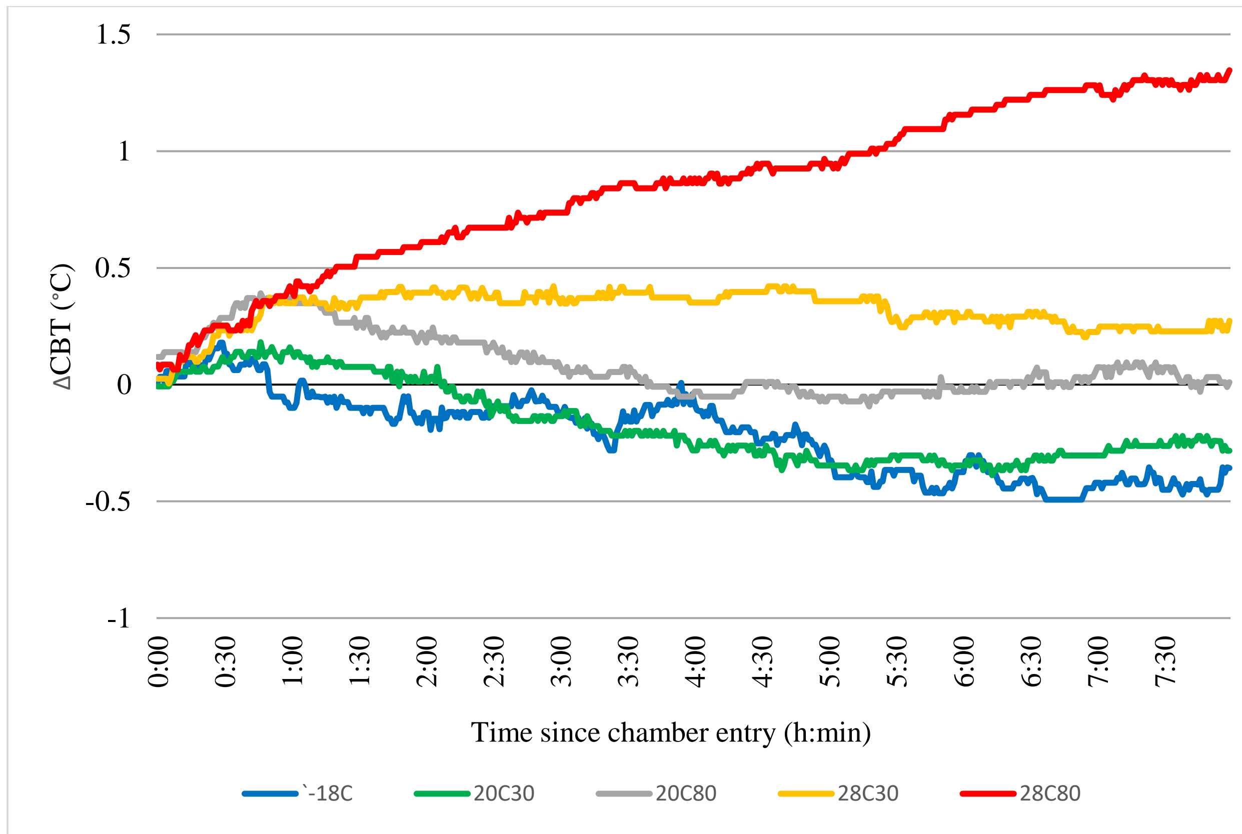


Figure 6.1: Mean tom ΔCBT when exposed to -18°C, 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, or 28°C 80% RH for 8 hours, using mean individual CBT during the last 30 minutes of lairage as a baseline.

The shivering and huddling observed in the -18°C treatments, more frequent in hens, indicated that there was a need to alter behaviour to cope with cold conditions. Toms also employed such behaviours during cold-exposure, and tended to ptiloerect more than hens. Size appeared to be an important factor in thermal coping, with the smaller, younger hens experiencing more pronounced responses to cold temperatures than toms, both physiologically and behaviourally. There was some indication of hypothermia among cold-exposed hens, with trends of ΔCBT over time (Figure 6.2) appearing to support this (no statistical analysis was performed). However, it is worth noting that in 4 of 5 treatments, hen CBT had settled below the

half-hour lairage baseline by the end of the exposure period. All hens ΔCBT values showed a similar pattern of rise above, and then fall below, the established baseline. Circadian rhythmicity of heat production has been demonstrated in broilers and turkeys, which may explain this consistent variation, though it was not apparent in toms (Macleod et al., 1985; Koh and Macleod, 1999). In general, turkeys were robust in the face of cold exposure, and anecdotally, were alert and quick to recover after removal from the climate-controlled chamber. The larger size of toms likely contributed to both their resistance to cold and increased susceptibility to warm temperatures, as it provides relatively less surface area from which to lose heat as well as a greater capacity for heat production. Overall, turkeys appeared more susceptible to warm exposure than broilers, particularly the larger toms, but even the hens – neither sex was able to satisfactorily withstand the previously selected (and rejected) 35°C temperatures, as discussed by Vermette C.J. et al. in a yet-to-be published article (University of Saskatchewan, Saskatoon, SK, personal communication).

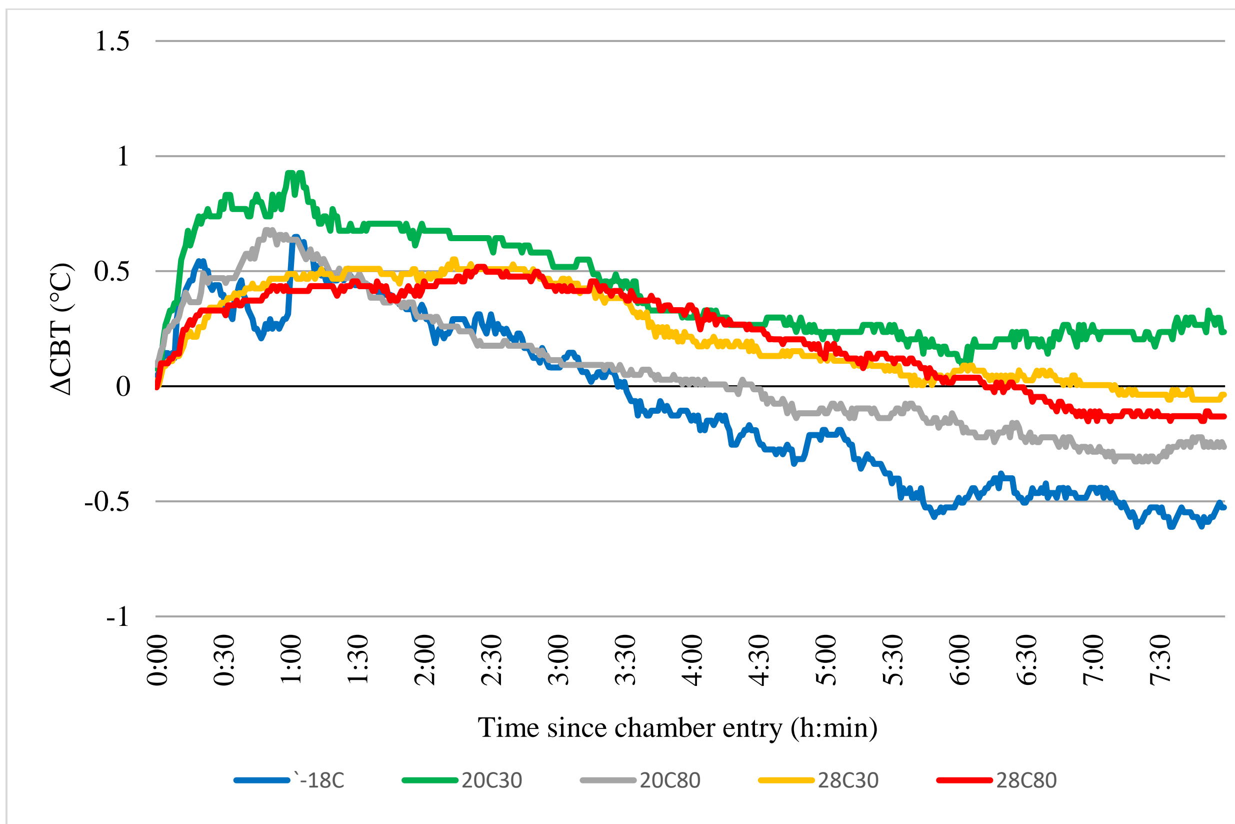


Figure 6.2: Mean hen ΔCBT when exposed to -18°C, 20°C 30% RH, 20°C 80% RH, 28°C 30% RH, or 28°C 80% RH for 8 hours, using mean individual CBT during the last 30 minutes of lairage as a baseline.

While the heterophil-lymphocyte ratio has often been relied on to make inferences about both thermal stress and welfare in poultry (Gross, 1988; Altan et al., 2003; Warriss et al., 2005), no effect of cold exposure was detected, and only toms in the warm, high-humidity treatment had a tendency for a higher HLR. The lack of significance in this measure may have been related to the relatively short exposure duration and immediate subsequent sampling, as changes to HLR do not respond instantly to stressors, instead taking several hours to develop. Additionally, the lack of pre-exposure baseline data with which to compare and high individual variability limited the usefulness of the data. In future research, obtaining baseline data is recommended, as is consideration of corticosterone as a more rapidly-responsive measure of environmental stress. Despite the lack of significant HLR findings, some conclusions may be drawn regarding bird welfare based on behavioural and physiological responses. As noted, thermal stress was not extreme, and the changes in CBT and live shrink were not sufficient to conclude that major detriment to health or welfare had occurred – though they may have been exposed to hunger and thirst beyond that of turkeys transported at near thermoneutral temperatures. Turkeys exposed to warm and cold conditions did however display signs of discomfort and stress, and significantly altered their behaviour to cope with the environment. While not likely in severe distress, welfare was compromised by exposure to these transport conditions.

Several impacts on meat quality by temperature and humidity were noted, though neither PSE meat due to heat stress nor DFD meat due to cold stress were observed. After cold exposure, the smaller, more vulnerable hens displayed changes to initial and ultimate muscle pH and minor effects on meat colour, while the larger toms were largely unaffected. Fortunately, these minimal impacts are not considered likely to result in consumer rejection nor compromise meat quality, although processors may wish to sort affected cuts for ease of processing and packaging (Fernandez et al., 2002). Warm exposure, on the other hand, impacted the meat quality of hens and toms in different ways. Hens were less strongly affected, with changes in muscle pH not persisting beyond initial measurements, and not decreasing as expected in heat-stressed poultry. Though they did display colour changes associated with warm exposure, including lighter breast meat, the pH findings negated the possibility of PSE and the likelihood of consumer rejection or meat quality detriment (Froning et al., 1978; Owens et al., 2000). Warm-exposed toms, despite experiencing physiological changes indicative of heat stress, did not have widespread or dramatic changes to meat quality. Unlike hens, no colour changes were observed, but pH

differences in accordance with heat stress persisted into ultimate measurements and thus the final meat product. While this finding may be an early indicator of PSE-type changes, it was not severe enough to suggest the defect was occurring nor compromise meat quality. Of note, the breast redness value of toms was numerically higher than that of hens across treatments, though only significantly so in the 20°C 80% RH condition. This difference may be explained by inherent sex-related variation, though it is difficult to determine due to the age and size differences between hens and toms in this study.

Change in blood glucose over the treatment duration also differed between sexes. In both 20°C conditions, hens experienced a significantly greater drop in blood glucose than toms, and this pattern was continued, albeit insignificantly, in the 28°C treatments. While all transported birds experience such a drop due to both feed withdrawal and the metabolic demands of the transport process, the smaller hens may have expended relatively more of their energy reserves coping with the same conditions than the larger toms. Oddly, this pattern was reversed during cold exposure, where toms experienced a larger drop in blood glucose – an unexpected result considering they experienced less live shrink and had fewer changes to meat quality. Further understanding of the response of blood glucose to various transport conditions, and changes in that response with differing sex, age, and size, may help to explain this finding.

In the present study, the exposure temperatures of -18°C and 28°C did not cause excessive thermal stress, but cold-exposed hens and warm-exposed toms began to show signs that their thermoregulatory abilities were being exceeded. These exposure conditions likely caused some detriment to welfare, and the impacts on live shrink and meat quality are worthy of consideration from a yield and meat processing standpoint. Further research into the responses of hens and toms, and the characteristics driving their differences, will help to guide transport recommendations. Additional research both in and out of the field will improve understanding of the impacts of more extreme temperatures, moisture and humidity, wind speed, and other transport stressors, and allow for the determination of acceptable thresholds. The benefits of improving conditions during transport are more than just altruistic, and serve to meet consumer demands and improve productivity. Reducing loss or waste due to consumer rejection, condemnations, live shrink, and DOA's are all potential benefits of a comprehensive understanding of turkeys' responses to adverse transport conditions.

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APPENDICES

Appendix A: Raw physiological and meat quality data

Table A1: Tom physiological data

tom	flock	pen	trt	temp	RH	ibutton	correct CBT	initial glucose	final glucose	initial wt	final wt	% live shrink	Avg HL
49	A	1	T1	20	30	203x	41.94	11.9	13.7	16.90	16.60	1.78	3.21
50	A	1	T1	20	30	245x	40.50	12.1	13.4	16.69	16.09	3.59	0.90
51	A	1	T1	20	30	227x	41.72	13.6	13.3	14.56	14.50	0.41	1.78
52	A	1	T1	20	30	276x	41.57	16.2	14.4	17.50	17.00	2.86	3.80
53	A	1	T1	20	30	213x	41.27	14.3	13.6	16.30	15.90	2.45	1.61
54	A	1	T1	20	30	230x	41.40	15.2	13.5	15.00	15.10	-0.67	1.96
55	A	1	T1	20	30	248x	41.57	12.8	12.2	15.60	15.00	3.85	1.23
56	A	1	T1	20	30	221x	.	14.3	15.5	16.95	16.60	2.06	2.31
65	A	2	T2	20	80	264x	41.58	13.5	12.6	18.16	17.79	2.04	1.98
66	A	2	T2	20	80	203x	41.07	14.8	14.1	16.07	15.77	1.87	1.79
67	A	2	T2	20	80	280x	40.62	13.9	12.9	18.01	17.70	1.72	1.53
68	A	2	T2	20	80	213x	40.95	14.1	14.3	16.99	16.70	1.71	1.33
69	A	2	T2	20	80	218x	41.49	14.8	12.5	17.70	17.20	2.82	0.89
70	A	2	T2	20	80	276x	41.09	13.8	14	15.87	15.59	1.76	0.87
71	A	2	T2	20	80	226x	41.06	13.9	12.3	17.75	17.36	2.20	1.70
72	A	2	T2	20	80	215x	41.00	13.8	13.4	15.75	15.38	2.35	0.82
73	A	3	T3	-18	.	245x	40.50	17.6	14.7	16.54	16.28	1.52	2.27
74	A	3	T3	-18	.	221x	.	15.7	12.2	15.38	15.05	2.15	1.73
75	A	3	T3	-18	.	253x	40.13	15.2	10.9	18.13	17.83	1.66	2.12
76	A	3	T3	-18	.	237x	40.96	15.6	16.2	16.33	16.06	1.66	3.54
77	A	3	T3	-18	.	248x	40.57	13.6	11.9	18.25	17.98	1.49	1.64
78	A	3	T3	-18	.	251x	40.03	16	12.9	17.01	16.77	1.42	3.72
79	A	3	T3	-18	.	229x	39.99	13.9	13.5	17.77	17.58	1.07	1.27
80	A	3	T3	-18	.	223x	40.78	16.6	14.1	15.26	15.03	1.51	1.58

137	B	1	T1	20	30	285x	41.38	15.4	14.9	15.74	15.42	2.04	1.44
138	B	1	T1	20	30	200x	41.33	15.2	15.6	16.54	16.34	1.15	1.60
139	B	1	T1	20	30	287x	41.11	14.8	11.7	18.53	18.25	1.52	1.38
140	B	1	T1	20	30	269x	42.49	16.9	15.7	15.50	15.36	0.91	2.38
141	B	1	T1	20	30	224x	41.08	15.2	14.2	16.57	16.29	1.64	2.40
142	B	1	T1	20	30	261x	41.83	13.7	14.1	17.44	17.14	1.73	1.97
143	B	1	T1	20	30	175x	41.41	15.6	14.6	15.73	15.63	0.64	3.22
144	B	1	T1	20	30	289x	40.49	13.1	12.1	17.49	17.24	1.44	1.07
153	B	2	T2	20	80	218x	41.49	14.5	13.7	16.19	15.93	1.61	1.23
154	B	2	T2	20	80	224x	40.72	14.3	15.7	15.85	15.59	1.65	1.03
155	B	2	T2	20	80	150x	41.22	15.2	15.2	16.40	16.20	1.22	1.44
156	B	2	T2	20	80	261x	41.20	13.6	12.7	17.09	16.87	1.29	2.23
157	B	2	T2	20	80	289x	40.44	13.9	12.9	16.92	16.60	1.90	0.95
158	B	2	T2	20	80	287x	41.61	15.6	13.2	14.89	14.65	1.62	1.31
159	B	2	T2	20	80	157x	41.63	13.3	13.9	16.05	15.86	1.19	1.40
160	B	2	T2	20	80	175x	41.23	13	12.6	16.98	16.69	1.71	2.48
169	B	3	T3	-18	.	287x	40.75	14	12.2	17.28	17.05	1.34	1.99
170	B	3	T3	-18	.	261x	40.82	14.6	12.6	17.42	17.11	1.79	1.74
171	B	3	T3	-18	.	187x	41.06	14.7	11.4	17.18	16.97	1.23	0.92
172	B	3	T3	-18	.	264x	40.85	15.1	11.2	16.17	15.86	1.92	1.23
173	B	3	T3	-18	.	269x	40.73	13	11	17.62	17.30	1.82	1.72
174	B	3	T3	-18	.	241x	41.19	14.5	12.3	15.53	15.32	1.36	1.15
175	B	3	T3	-18	.	285x	40.63	12.6	12.8	16.68	16.48	1.20	0.96
176	B	3	T3	-18	.	224x	40.63	14.5	12.9	16.03	15.74	1.82	2.12
233	C	1	T1	20	30	318x	41.07	15.6	13.6	18.22	18.06	0.88	1.44
234	C	1	T1	20	30	310x	41.77	13.5	14	17.42	17.23	1.10	1.06
235	C	1	T1	20	30	307x	40.03	14.3	13.8	13.26	13.10	1.21	1.39
236	C	1	T1	20	30	306x	40.88	15.3	13.2	15.11	14.87	1.59	1.57
237	C	1	T1	20	30	301x	41.18	14.9	12.9	16.00	15.76	1.51	1.54
238	C	1	T1	20	30	309x	41.68	14.6	16.4	15.41	15.16	1.63	1.14

239	C	1	T1	20	30	314x	42.10	14.8	14.7	15.83	15.50	2.09	1.70
240	C	1	T1	20	30	312x	40.62	15.8	13.8	17.00	16.80	1.18	1.67
249	C	2	T2	20	80	311x	39.49	16.6	13.6	17.28	17.15	0.76	0.80
250	C	2	T2	20	80	314x	42.31	17.6	13.5	15.05	14.94	0.73	1.33
251	C	2	T2	20	80	308x	40.80	17.7	13.6	18.04	17.85	1.06	1.05
252	C	2	T2	20	80	313x	41.96	15.7	14	15.72	15.58	0.89	1.17
253	C	2	T2	20	80	309x	41.75	17.7	13.7	16.02	15.97	0.31	1.07
254	C	2	T2	20	80	303x	41.79	17.3	14.8	16.36	16.20	0.98	1.03
255	C	2	T2	20	80	319x	40.85	15.8	14.3	16.56	16.32	1.39	1.10
256	C	2	T2	20	80	301x	41.18	15.5	14.8	16.74	16.66	0.48	1.28
265	C	3	T3	-18	.	309x	41.68	17.2	13.5	15.39	15.18	1.37	1.67
266	C	3	T3	-18	.	311x	40.21	16.8	13.6	17.06	16.77	1.71	1.77
267	C	3	T3	-18	.	316x	41.12	16.7	13.8	15.78	15.52	1.65	.
268	C	3	T3	-18	.	302x	40.78	16.7	13.4	16.47	16.24	1.34	.
269	C	3	T3	-18	.	308x	40.51	18.3	13	14.79	14.43	2.44	.
270	C	3	T3	-18	.	312x	.	16.4	12.8	15.34	15.22	0.79	.
271	C	3	T3	-18	.	310x	42.03	17.1	13.6	17.59	17.40	1.08	.
272	C	3	T3	-18	.	323x	41.12	16.8	13	16.76	16.51	1.50	.
129	B	4	T4	28	30	253x	41.59	16	15.6	16.21	15.81	2.48	1.37
130	B	4	T4	28	30	218x	41.99	16.3	14.2	16.86	16.41	2.62	1.56
131	B	4	T4	28	30	187x	42.23	16.4	12.4	17.74	17.21	3.00	1.89
132	B	4	T4	28	30	241x	42.20	13.9	15	17.81	17.22	3.33	1.07
133	B	4	T4	28	30	207x	41.18	15.6	12.3	14.34	14.00	2.38	1.52
134	B	4	T4	28	30	157x	42.32	14.5	12.3	16.68	16.18	2.95	3.01
135	B	4	T4	28	30	163x	.	14.6	14.2	16.20	15.79	2.54	2.17
136	B	4	T4	28	30	264x	42.12	14.5	13.1	15.34	15.10	1.57	1.89
145	B	5	T5	28	80	264x	42.06	13.5	12.4	14.90	14.62	1.89	1.78
146	B	5	T5	28	80	241x	42.20	15.7	12.9	15.19	14.89	1.98	1.84
147	B	5	T5	28	80	269x	41.74	13.9	13.6	13.49	13.11	2.83	1.07
148	B	5	T5	28	80	285x	41.64	13.6	13.2	15.76	15.45	1.97	1.60

149	B	5	T5	28	80	187x	42.07	13.2	13.5	17.41	16.91	2.88	1.87
150	B	5	T5	28	80	207x	42.51	16.2	14.3	14.65	14.38	1.85	1.55
151	B	5	T5	28	80	200x	41.83	13.1	13.2	15.90	15.44	2.90	1.29
152	B	5	T5	28	80	253x	42.87	13.1	13.6	17.61	17.11	2.85	2.03
161	B	4	T5	28	80	150x	41.57	15.5	13.7	17.22	17.00	1.28	0.88
162	B	4	T5	28	80	289x	40.99	15.2	12.2	17.04	16.67	2.18	1.71
163	B	4	T5	28	80	157x	43.69	13.9	12.7	17.68	17.14	3.07	2.65
164	B	4	T5	28	80	175x	41.76	14.5	13.1	16.26	15.88	2.35	1.09
165	B	4	T5	28	80	200x	41.79	16	14.4	15.45	14.98	3.05	2.42
166	B	4	T5	28	80	253x	40.89	15.5	13.3	16.71	16.26	2.64	1.08
167	B	4	T5	28	80	218x	41.99	13.4	13.3	18.79	18.41	2.03	2.28
168	B	4	T5	28	80	207x	41.50	13.4	13.2	18.05	17.58	2.61	0.95
225	C	4	T4	28	30	308x	41.51	14.6	15.2	15.79	15.30	3.12	1.60
226	C	4	T4	28	30	313x	42.47	13.3	13.6	15.71	15.30	2.62	1.34
227	C	4	T4	28	30	302x	41.13	14.6	14.1	15.62	15.20	2.70	1.41
228	C	4	T4	28	30	319x	42.32	12.7	14.6	16.10	15.59	3.18	1.35
229	C	4	T4	28	30	311x	41.14	13.9	14.6	14.44	13.92	3.62	0.99
230	C	4	T4	28	30	323x	41.96	13.6	14.4	15.05	14.68	2.47	1.29
231	C	4	T4	28	30	316x	42.13	12.4	14.2	16.21	15.64	3.53	1.65
232	C	4	T4	28	30	303x	42.38	13.9	12.6	16.44	16.08	2.14	1.04
257	C	5	T4	28	30	301x	41.69	15.6	14.3	16.56	16.24	1.88	0.96
258	C	5	T4	28	30	303x	41.88	15.1	16.1	15.66	15.31	2.24	1.20
259	C	5	T4	28	30	314x	43.15	14.8	14.8	17.67	17.21	2.61	1.35
260	C	5	T4	28	30	319x	41.82	14.6	14	15.49	15.13	2.33	1.13
261	C	5	T4	28	30	313x	41.94	17	13.1	15.88	15.55	2.09	1.67
262	C	5	T4	28	30	318x	41.54	14.4	15.8	17.10	16.59	2.99	0.98
263	C	5	T4	28	30	307x	40.54	16.2	13.8	16.93	16.62	1.84	1.29
264	C	5	T4	28	30	306x	41.19	14.9	13.8	16.16	15.75	2.55	1.31
241	C	5	T5	28	80	310x	44.49	13.2	14.6	15.59	15.23	2.32	2.52
242	C	5	T5	28	80	318x	42.39	17.7	15.5	15.43	15.00	2.80	1.39

243	C	5	T5	28	80	306x	45.36	15.7	17.2	16.57	15.97	3.58	.
244	C	5	T5	28	80	323x	42.89	14.2	15.6	15.78	15.60	1.15	2.56
245	C	5	T5	28	80	316x	45.29	14.9	17.2	17.61	17.29	1.82	7.27
246	C	5	T5	28	80	312x	45.38	15.7	18.1	17.32	16.89	2.49	.
247	C	5	T5	28	80	307x	42.52	15.4	16.1	17.37	16.95	2.43	4.24
248	C	5	T5	28	80	302x	43.66	15.8	16.4	18.41	18.01	2.18	2.45

Table A2: Tom meat quality data

tom	flock	pen	trt	temp	RH	initial pH BP	ult pH BP	initial pH BS	ult pH BS	pH thigh	breast L*	breast a*	breast b*	thigh L*	thigh a*	thigh b*
49	A	1	T1	20	30	.	.	6.64	5.61	.	52.5	3.63	-1.485	51.1	15.095	0.865
50	A	1	T1	20	30	.	.	6.36	5.64	.	51.875	4.37	-2.04	48.57	15.705	1.975
51	A	1	T1	20	30	.	.	6.49	5.69	.	48.35	5.365	-1.83	51.845	14.475	2.18
52	A	1	T1	20	30	.	.	6.51	5.79	.	47.01	6.515	-0.965	52.635	12.31	0.825
53	A	1	T1	20	30	.	.	6.46	5.58	.	50.73	5.08	-1.815	50.245	14.97	1.725
54	A	1	T1	20	30	.	.	6.41	.	.	48.72	5.115	-2.39	51.215	11.365	-1.51
55	A	1	T1	20	30	.	.	6.37	5.63	.	49.65	10.7	0.51	50.37	9.415	-0.645
56	A	1	T1	20	30	.	.	6.43	5.75	.	47.765	5.45	-2.83	44.725	17.93	0.95
65	A	2	T2	20	80	7.04	5.6	6.76	.	5.79	49.78	4.545	-2.62	41.195	13.28	-3.765
66	A	2	T2	20	80	6.91	5.67	6.42	5.69	5.85	48.535	4.67	-1.72	47.295	11.645	-3.95
67	A	2	T2	20	80	6.74	5.65	6.36	5.69	5.85	50.92	4.865	-0.47	51.625	13.17	0.77
68	A	2	T2	20	80	6.96	5.68	6.57	5.59	5.63	50.925	4.86	-2.155	48.345	12.325	-1.43
69	A	2	T2	20	80	6.8	5.7	6.49	5.65	6.02	50.845	5.095	-0.945	46.525	11.83	0.385
70	A	2	T2	20	80	6.6	5.65	6.34	.	5.83	48.93	4.95	-3.125	50.39	14.12	-0.74
71	A	2	T2	20	80	6.3	5.69	6.57	5.74	5.75	51.1	4.64	-2.415	50.58	13.195	-0.17
72	A	2	T2	20	80	6.78	5.73	6.43	5.82	5.75	36.82	7.015	-9.67	47.875	10.025	-3.175
73	A	3	T3	-18	.	6.56	5.77	6.6	.	6.08	50.53	4.175	-1.495	45.455	11.68	-1.73
74	A	3	T3	-18	.	6.28	5.62	6.78	5.69	6.2	51.495	3.89	-1.51	45.935	13.075	0.605

75	A	3	T3	-18	.	6.85	5.71	6.71	5.63	5.91	48.02	13.73	-0.715	50.5	4.125	-2.115
76	A	3	T3	-18	.	6.63	5.66	6.32	5.72	5.97	50.3	4.185	-1.835	50.455	8.965	-1.79
77	A	3	T3	-18	.	6.57	5.68	6.6	.	6.01	50.5	4.125	-2.115	47.05	10.155	-3.605
78	A	3	T3	-18	.	6.63	5.85	6.71	6.03	6.23	45.445	4.76	-3.18	47.46	13.77	-2.92
79	A	3	T3	-18	.	6.95	5.74	6.57	5.79	5.96	51.65	3.285	-1.495	50.38	13.01	-2.46
80	A	3	T3	-18	.	6.44	5.71	6.44	5.71	5.95	52.88	4.32	-1.605	47.845	13.14	-1.33
137	B	1	T1	20	30	6.76	5.74	6.61	5.79	5.72	48.875	5.195	-4.12	49.475	15.235	-2.145
138	B	1	T1	20	30	6.68	5.65	6.55	5.75	5.81	48.52	4.93	-3.205	51.525	15.375	2.485
139	B	1	T1	20	30	6.97	5.78	6.51	5.77	5.79	49.875	5.43	-1.855	48.495	15.52	-1.295
140	B	1	T1	20	30	6.85	5.76	6.74	5.78	5.88	47.895	6.11	-2.96	55.645	14.65	0.335
141	B	1	T1	20	30	6.74	5.77	6.67	5.83	5.97	52.5	2.78	-3.53	54.36	12.54	-0.675
142	B	1	T1	20	30	6.9	5.67	6.54	5.74	5.93	51.14	5.535	-2.215	45.375	11.685	-2.605
143	B	1	T1	20	30	6.82	5.71	6.5	5.75	5.96	51.6	3.595	-3.01	38.73	11.545	-3.2
144	B	1	T1	20	30	7.07	5.63	6.63	5.72	5.74	54.45	4.755	-1.805	51.33	15.375	0.295
153	B	2	T2	20	80	6.87	5.69	6.18	5.71	6.12	49.72	5.345	-2.12	46.965	19.615	3.02
154	B	2	T2	20	80	6.6	5.65	6.43	5.66	6.02	50.515	4.985	-2.62	47.435	16.15	0.855
155	B	2	T2	20	80	6.56	5.77	6.6	5.68	5.86	51.75	5.155	-2.68	54.36	14.805	1.57
156	B	2	T2	20	80	6.89	5.69	6.56	5.68	5.97	52.3	6.28	-1.215	50.68	17.46	3.485
157	B	2	T2	20	80	6.84	5.71	6.71	5.72	5.95	51.185	5.265	-3.055	50.155	15.08	1.17
158	B	2	T2	20	80	6.72	5.79	6.29	5.81	6.03	49.35	4.92	-4.24	53.77	15.17	2.645
159	B	2	T2	20	80	6.63	5.65	6.46	5.79	5.72	48.505	6.12	-3.565	47.035	14.935	-1.415
160	B	2	T2	20	80	6.42	5.72	6.24	5.82	5.96	48.305	6.875	-1.78	48.23	16.93	1.445
169	B	3	T3	-18	.	6.63	5.71	6.57	5.6	6	51.1	5.78	-0.875	45.765	19.595	2.965
170	B	3	T3	-18	.	6.78	5.73	6.4	5.76	6.07	51.56	4.83	-2.545	50.96	15.205	2.215
171	B	3	T3	-18	.	6.86	5.73	6.22	5.78	6.13	51.855	3.885	-1.27	51.81	13.48	0.7
172	B	3	T3	-18	.	6.76	5.71	6.5	5.7	6.01	50.285	5.505	-2.295	50.195	15.305	1.85
173	B	3	T3	-18	.	6.92	5.66	6.39	5.68	6.03	54.57	4.655	-2.01	42.755	17.245	0.03
174	B	3	T3	-18	.	6.36	5.77	6.28	5.74	6.07	49.98	4.51	-2.94	49.8	16.165	1.19

175	B	3	T3	-18	.	6.7	5.66	6.44	5.61	6.15	53.125	4.505	-1.525	49.91	14.24	0.18
176	B	3	T3	-18	.	6.86	5.76	6.43	5.69	6.13	50.055	4.635	-3.005	48.865	14.07	1.02
233	C	1	T1	20	30	6.36	5.75	6.36	5.73	5.88	50.605	8.625	-1.695	52.89	14.395	1.305
234	C	1	T1	20	30	6.59	5.71	6.33	5.65	5.84	50.085	8.01	-2.135	50.455	8.18	-1.96
235	C	1	T1	20	30	6.81	5.69	6.42	5.63	5.82	51.1	8.13	-2.395	50.12	8.86	-2.28
236	C	1	T1	20	30	6.75	5.76	6.3	5.69	5.82	51.79	7.85	-3.035	52.815	8.75	-1.135
237	C	1	T1	20	30	6.89	5.74	6.32	5.68	5.83	49.265	19.905	0.345	50.72	11.495	-1.22
238	C	1	T1	20	30	6.63	5.69	6.24	5.66	5.75	52.115	3.98	-2.585	47.52	14.125	-0.445
239	C	1	T1	20	30	6	5.66	6.06	5.56	5.71	51.945	7.885	-2.11	51.29	7.88	-2.08
240	C	1	T1	20	30	6.86	5.69	6.36	5.62	5.87	50.965	4.575	-3.68	53.435	10.29	-1.555
249	C	2	T2	20	80	6.81	5.71	6.47	5.65	5.85	49.22	5.555	-2.59	50.2	15.055	2.075
250	C	2	T2	20	80	6.8	5.63	6.28	5.64	5.93	48.125	6.14	-3.565	48.84	15.055	0.13
251	C	2	T2	20	80	6.94	5.66	6.39	5.53	5.86	54.66	7.075	-0.435	51.83	7.925	-1.905
252	C	2	T2	20	80	6.83	5.85	6.42	5.69	5.82	51.265	4.52	-2.9	49.335	18.08	4.36
253	C	2	T2	20	80	6.81	5.69	6.39	5.75	5.66	52.185	5.915	-1.87	51.265	15.52	2.79
254	C	2	T2	20	80	6.56	5.76	6.29	5.8	5.78	49.915	4.645	-3.495	47.995	13.7	0.82
255	C	2	T2	20	80	6.87	5.6	6.29	5.74	5.92	52.12	4.635	-2.33	58.66	9.17	2.1
256	C	2	T2	20	80	6.99	5.62	6.35	5.77	6.01	53.285	8.35	-0.425	52.15	9.14	-0.145
265	C	3	T3	-18	.	6.85	5.7	6.47	5.65	5.88	48.57	15.705	1.975	52.5	3.63	-1.485
266	C	3	T3	-18	.	6.66	5.75	6.39	5.69	6.01	48.715	10.855	-0.085	47.065	12.52	-0.555
267	C	3	T3	-18	.	6.98	5.79	6.43	5.62	6.15	46.81	15.38	-1.2	49.58	6.455	-2.125
268	C	3	T3	-18	.	6.47	5.74	6.32	5.74	6.28	51.27	8.285	-1.83	50.205	9.295	-0.2
269	C	3	T3	-18	.	6.93	5.79	6.38	5.76	6.09	46.86	14.585	-1.315	46.965	5.155	-4.435
270	C	3	T3	-18	.	6.62	5.82	6.42	5.77	6.28	46.635	18.255	-0.26	51.875	4.37	-2.04
271	C	3	T3	-18	.	6.77	5.63	6.45	5.63	6.11	45.5	15.485	0.87	49.04	3.81	-2.495
272	C	3	T3	-18	.	6.33	5.88	6.4	5.84	6.33	51.1	15.095	0.865	50.02	8.245	-1.955
129	B	4	T4	28	30	6.78	.	6.66	5.8	5.78	51.61	4.485	-3.43	50.355	15.585	2
130	B	4	T4	28	30	6.78	5.67	6.7	5.79	6	51.345	4.565	-2.835	52.065	14.495	1.085

131	B	4	T4	28	30	6.66	5.76	6.56	5.77	5.88	52.78	5.17	-1.795	49.45	15.38	0.745
132	B	4	T4	28	30	6.81	5.73	6.69	5.77	5.91	51.55	6.14	-4.175	46.455	18.81	-1.035
133	B	4	T4	28	30	6.72	5.68	6.56	5.73	5.54	48.49	6.215	-2.69	45.75	14.865	-1.085
134	B	4	T4	28	30	6.83	5.88	6.43	5.88	5.97	51.13	4.135	-4.205	49.175	16.265	1.555
135	B	4	T4	28	30	6.73	5.86	6.56	5.81	5.96	47.525	6.615	-3.975	48.795	16.35	2.145
136	B	4	T4	28	30	6.94	5.45	6.49	5.81	5.68	50.37	6.59	-1.93	52.88	14.275	2.05
145	B	5	T5	28	80	6.84	5.72	6.78	5.74	5.9	46.11	5.82	-3.14	50.225	13.815	0.265
146	B	5	T5	28	80	6.5	6.03	6.62	6.08	6.11	45.28	3.705	-3.69	51.315	15.205	1.92
147	B	5	T5	28	80	6.78	5.75	6.63	5.79	6.04	49.535	5.19	-3.025	52.965	13.47	0.71
148	B	5	T5	28	80	6.72	5.7	6.64	5.71	6.15	48.84	5.62	-3.695	49.625	15.82	2.07
149	B	5	T5	28	80	6.38	5.58	6.61	5.56	5.91	52.035	5.72	-2.165	48.205	16.88	1.71
150	B	5	T5	28	80	6.98	5.62	6.44	5.6	5.97	51.115	5.075	-3.165	47.325	12.695	-2.755
151	B	5	T5	28	80	6.7	5.62	6.42	5.67	6.01	52.185	5.065	-0.95	51.985	14.78	1.55
152	B	5	T5	28	80	6.39	5.73	6.26	5.7	5.61	49.205	5.63	-3.195	48.69	16.06	-0.09
161	B	4	T5	28	80	7.04	5.76	6.39	5.75	6.04	51.49	4.025	-3.225	52.665	14.29	1.725
162	B	4	T5	28	80	6.84	5.7	6.3	5.63	6.03	55.095	5.01	-3.46	52.045	14.825	2.03
163	B	4	T5	28	80	6.65	5.72	6.35	5.69	5.87	51.765	6.335	-1.69	50.53	20.29	3.49
164	B	4	T5	28	80	6.78	5.78	6.46	5.72	5.96	53.59	4.25	-1.88	51.55	17.11	3.835
165	B	4	T5	28	80	6.75	5.94	6.43	5.81	5.96	45.505	5.905	-3.015	50.32	15.09	2.65
166	B	4	T5	28	80	6.7	5.79	6.46	5.75	6.1	49.71	4.035	-4.01	49	16.22	2.905
167	B	4	T5	28	80	6.92	5.77	6.45	5.75	5.86	57.06	5.15	-2.28	50.315	15.015	-0.105
168	B	4	T5	28	80	6.65	5.78	6.55	5.68	5.81	52.01	4.51	-2.47	52.19	16.46	2.965
225	C	4	T4	28	30	6.89	5.72	6.28	5.69	5.89	53.11	8.52	-0.265	47.795	12.365	-0.565
226	C	4	T4	28	30	6.83	5.72	6.35	5.7	6	48.71	12.655	-0.98	54.175	3.865	-1.565
227	C	4	T4	28	30	6.89	5.72	6.29	5.7	5.96	49.95	9.78	-0.89	51.76	9.165	-0.74
228	C	4	T4	28	30	6.64	5.67	6.26	5.62	5.7	52.385	9.19	-1.68	51.395	9.215	-1.675
229	C	4	T4	28	30	6.5	5.75	6.25	5.72	5.65	51.485	9.73	0.23	51.875	10.14	0.19
230	C	4	T4	28	30	6.91	5.76	6.17	5.75	5.97	48.195	8.21	0.1	50.295	6.09	-2

231	C	4	T4	28	30	7.01	5.77	6.34	5.71	5.83	53.325	3.905	-1.18	45.665	13.725	-2.805
232	C	4	T4	28	30	6.89	5.71	6.3	5.6	5.89	53.805	3.85	-2.345	51.55	10.025	1.615
257	C	5	T4	28	30	6.81	5.69	6.63	5.76	5.76	46.88	16.73	0.875	48.1	5.07	-3.11
258	C	5	T4	28	30	6.71	5.78	6.5	5.8	5.82	47.985	11.415	-1.85	45.675	4.895	-4.38
259	C	5	T4	28	30	6.67	5.81	6.53	5.73	5.94	50.35	4.615	-3.195	46.68	10.305	-1.465
260	C	5	T4	28	30	6.87	5.72	6.58	5.68	5.87	51.82	5.03	-2.215	51.235	11.165	0.52
261	C	5	T4	28	30	6.52	5.69	6.26	5.63	5.87	47.58	10.405	-1.18	51.06	8.655	-0.08
262	C	5	T4	28	30	6.64	5.68	6.44	5.62	5.83	50.21	4.33	-3.885	47.95	4.435	-3.475
263	C	5	T4	28	30	6.73	5.69	6.42	5.6	5.85	40.305	17.68	-2.11	45.87	5.025	-4.38
264	C	5	T4	28	30	6.85	5.76	6.49	5.66	5.78	50.255	12.85	-0.02	49.17	4.55	-3.395
241	C	5	T5	28	80	6.98	5.66	6.5	5.6	5.73	50.55	9.17	-1.03	50.84	7.25	-2.03
242	C	5	T5	28	80	6.75	5.76	6.44	5.74	5.75	50.07	8.445	-1.245	52.4	8.85	-2.04
243	C	5	T5	28	80	6.62	5.93	6.48	5.72	5.87	52.19	4.17	-3.095	49.635	12.845	-0.47
244	C	5	T5	28	80	6.54	5.67	6.25	5.6	5.8	51.13	8.305	-3.395	49.225	8.275	-2.675
245	C	5	T5	28	80	7	5.67	6.48	5.54	5.75	54.105	9.82	0.915	50.99	9.63	-0.465
246	C	5	T5	28	80	6.51	5.74	6.47	5.56	5.73	49.605	8.43	-1.725	49.165	9.515	-1.385
247	C	5	T5	28	80	6.45	5.83	6.38	5.53	5.85	48.905	8.065	-1.79	50.255	7.54	-0.73
248	C	5	T5	28	80	7.01	5.69	6.49	5.65	6.06	49.615	9.17	-2.55	50.09	9.265	-3.11

Table A3: Hen physiological data

hen	flock	pen	trt	temp	RH	ibutton	correct CBT	initial glucose	final glucose	correct initial wt	correct final wt	correct % live shrink	Avg HL
9	A	1	T1	20	30	264x	.	18.1	15.4	6.84	6.75	1.38	1.79
10	A	1	T1	20	30	280x	.	17.2	14.9	7.59	7.44	1.96	1.28
11	A	1	T1	20	30	248x	.	16.7	15.1	6.94	6.85	1.37	3.53
12	A	1	T1	20	30	276x	.	19.5	13.1	6.49	6.45	0.65	1.48
13	A	1	T1	20	30	251x	.	17.1	13.3	7.34	7.24	1.33	1.81
14	A	1	T1	20	30	269x	.	15.3	12.7	6.89	6.85	0.65	1.54

15	A	1	T1	20	30	245x	.	18.4	15.2	6.59	6.35	3.66	0.91
16	A	1	T1	20	30	253x	.	14.5	12.3	6.84	6.75	1.38	2.39
25	A	2	T2	20	80	264x	41.25	14.2	12.9	7.29	7.15	2.01	1.85
26	A	2	T2	20	80	229x	41.10	14.6	13.9	6.14	6.05	1.46	2.11
27	A	2	T2	20	80	223x	41.56	15.8	14.4	6.54	6.45	1.41	4.55
28	A	2	T2	20	80	221x	.	14.6	14.1	7.14	7.05	1.35	1.02
29	A	2	T2	20	80	251x	40.96	15.6	12.5	7.04	7.05	-0.05	1.36
30	A	2	T2	20	80	248x	41.35	14.7	13.7	7.59	7.44	1.96	1.59
31	A	2	T2	20	80	245x	40.52	15.8	13.2	6.64	6.45	2.89	1.89
32	A	2	T2	20	80	269x	41.26	15.6	13.7	7.24	7.15	1.34	1.39
33	A	3	T3	-18	.	215x	40.78	15.8	13.6	7.64	7.44	2.60	1.30
34	A	3	T3	-18	.	276x	41.20	13.6	11.1	7.14	6.95	2.74	1.32
35	A	3	T3	-18	.	251x	41.10	15.3	11.7	7.04	6.95	1.36	2.49
36	A	3	T3	-18	.	203x	41.12	15.2	13.2	6.19	5.86	5.46	0.57
37	A	3	T3	-18	.	253x	40.64	13.5	11.7	7.64	7.44	2.60	2.57
38	A	3	T3	-18	.	223x	41.01	16.4	13.2	7.79	7.54	3.20	1.14
39	A	3	T3	-18	.	269x	41.50	14.1	13.3	6.24	6.05	3.03	1.33
40	A	3	T3	-18	.	229x	40.00	16.3	12.4	6.69	6.45	3.62	1.10
89	B	1	T1	20	30	208x	41.03	15.6	14.41	7.22	7.15	0.97	1.74
90	B	1	T1	20	30	182x	41.57	14.7	13.6	7.00	6.92	1.15	1.86
91	B	1	T1	20	30	193x	41.85	15.4	12.7	8.27	8.17	1.21	1.21
92	B	1	T1	20	30	204x	41.46	15.2	14.8	8.06	7.94	1.49	1.34
93	B	1	T1	20	30	161x	41.72	14.9	14.4	7.53	7.49	0.53	1.19
94	B	1	T1	20	30	171x	.	16.8	13.8	7.57	7.47	1.33	0.98
95	B	1	T1	20	30	175x	41.15	15	13.8	7.66	7.59	0.92	1.97
96	B	1	T1	20	30	197x	42.28	17.2	15.9	7.95	7.87	1.01	1.50
105	B	2	T2	20	80	166x	41.84	14.6	12.6	7.68	7.59	1.18	1.23
106	B	2	T2	20	80	197x	41.67	14.8	12.8	7.74	7.66	1.04	1.36
107	B	2	T2	20	80	182x	41.10	15.4	13.9	7.62	7.52	1.32	0.99
108	B	2	T2	20	80	187x	41.32	14.5	13.1	7.82	7.72	1.28	1.29

109	B	2	T2	20	80	193x	41.41	13.5	14.3	6.92	6.87	0.73	1.02
110	B	2	T2	20	80	171x	.	13.4	12.4	8.17	8.07	1.23	2.62
111	B	2	T2	20	80	208x	40.54	16.3	13.3	7.51	7.37	1.87	1.86
112	B	2	T2	20	80	204x	41.22	13.9	13.4	7.83	7.67	2.05	1.77
121	B	3	T3	-18	.	173x	41.32	14.9	13.9	7.57	7.38	2.52	1.65
122	B	3	T3	-18	.	204x	40.97	16.9	12.3	7.80	7.60	2.57	1.53
123	B	3	T3	-18	.	151x	39.96	15.4	12.1	8.18	8.00	2.21	1.42
124	B	3	T3	-18	.	187x	41.16	14.5	13.9	6.99	6.84	2.16	1.21
125	B	3	T3	-18	.	198x	40.58	15.6	11.9	8.10	7.76	4.21	1.94
126	B	3	T3	-18	.	161x	41.58	15.9	12.7	7.87	7.66	2.68	1.82
127	B	3	T3	-18	.	171x	.	16.7	12.3	6.92	6.77	2.18	1.78
128	B	3	T3	-18	.	193x	41.24	15.1	12.2	7.54	7.27	3.60	1.40
185	C	1	T1	20	30	285x	41.12	15.3	14.4	7.69	7.49	2.61	1.17
186	C	1	T1	20	30	224x	41.28	17.1	15.7	7.11	6.95	2.26	1.06
187	C	1	T1	20	30	157x	41.70	18.7	15.6	7.22	7.09	1.81	1.28
188	C	1	T1	20	30	207x	41.05	17.7	15.4	6.63	6.49	2.12	1.56
189	C	1	T1	20	30	264x	41.11	15.7	11.5	7.66	7.62	0.52	1.39
190	C	1	T1	20	30	187x	41.57	15.7	14.4	7.56	7.46	1.33	1.73
191	C	1	T1	20	30	253x	40.65	15.4	13.8	7.35	7.26	1.23	1.30
192	C	1	T1	20	30	200x	41.15	19.2	15.3	7.53	7.39	1.87	1.02
201	C	2	T2	20	80	224x	40.43	14.6	12.4	8.45	8.28	2.02	1.13
202	C	2	T2	20	80	253x	40.12	14.3	13.5	7.45	7.37	1.08	0.78
203	C	2	T2	20	80	264x	41.12	16.4	13.2	8.55	8.38	2.00	1.41
204	C	2	T2	20	80	150x	40.89	15.7	12.4	7.55	7.47	1.06	1.41
205	C	2	T2	20	80	285x	41.14	14.6	14.1	7.19	7.09	1.40	1.39
206	C	2	T2	20	80	207x	40.64	14.2	12.8	7.59	7.49	1.32	1.59
207	C	2	T2	20	80	289x	40.45	18.6	14.3	7.83	7.71	1.54	1.28
208	C	2	T2	20	80	157x	41.46	15.2	14.4	7.25	7.14	1.52	1.40
217	C	3	T3	-18	.	207x	40.47	13.7	13.6	8.02	7.82	2.50	4.33
218	C	3	T3	-18	.	264x	41.10	14.1	14	7.38	7.22	2.18	1.24

219	C	3	T3	-18	.	261x	41.72	17.2	12.3	7.32	7.11	2.88	1.28
220	C	3	T3	-18	.	157x	41.85	14.8	11.2	8.17	7.88	3.56	1.71
221	C	3	T3	-18	.	269x	41.61	15.4	14.8	7.14	6.99	2.11	1.95
222	C	3	T3	-18	.	187x	40.63	15.1	12.6	7.40	7.28	1.63	1.29
223	C	3	T3	-18	.	224x	40.99	17.2	12.9	7.50	7.31	2.54	2.86
224	C	3	T3	-18	.	218x	41.23	14.8	14.2	7.34	7.14	2.74	0.95
81	B	4	T4	28	30	192x	40.99	19.6	16.2	7.63	7.39	3.16	1.09
82	B	4	T4	28	30	151x	40.95	19.5	15.4	7.87	7.66	2.68	1.52
83	B	4	T4	28	30	150x	41.72	15.8	14.3	7.45	7.21	3.23	1.31
84	B	4	T4	28	30	187x	42.07	16.4	13.3	7.19	6.98	2.93	5.82
85	B	4	T4	28	30	166x	42.35	16.3	16.3	8.05	7.82	2.87	2.13
86	B	4	T4	28	30	198x	41.67	16.7	14.2	6.66	6.55	1.66	3.11
87	B	4	T4	28	30	210x	41.81	15	13.6	6.97	6.80	2.45	0.96
88	B	4	T4	28	30	173x	41.77	14.9	14.5	7.67	7.46	2.75	2.28
97	B	5	T5	28	80	210x	41.74	16.8	13.3	7.59	7.39	2.65	2.36
98	B	5	T5	28	80	161x	42.54	16.1	13.6	8.29	7.94	4.24	1.37
99	B	5	T5	28	80	175x	41.41	15.9	15.3	7.89	7.60	3.69	1.47
100	B	5	T5	28	80	150x	41.86	14.4	14.7	7.13	7.00	1.83	1.66
101	B	5	T5	28	80	198x	41.57	15.4	13.2	8.08	7.85	2.86	1.68
102	B	5	T5	28	80	151x	40.95	14.8	13.9	7.93	7.69	3.04	2.07
103	B	5	T5	28	80	173x	41.46	16.3	15.3	7.36	7.12	3.27	1.22
104	B	5	T5	28	80	192x	41.43	15.5	14.7	6.81	6.63	2.65	1.45
113	B	4	T4	28	30	166x	42.28	13.6	14.1	7.25	7.15	1.39	0.93
114	B	4	T4	28	30	150x	41.72	14	14.4	7.17	7.10	0.98	1.19
115	B	4	T4	28	30	208x	41.25	15.4	15.2	6.06	5.91	2.48	1.00
116	B	4	T4	28	30	210x	41.74	15.2	12.3	7.21	7.07	1.95	1.89
117	B	4	T4	28	30	197x	42.09	15.9	14.6	7.16	6.91	3.51	1.59
118	B	4	T4	28	30	182x	41.57	14.3	12.3	7.89	7.67	2.80	1.18
119	B	4	T4	28	30	175x	40.91	14.3	12.4	8.18	7.94	2.94	1.19
120	B	4	T4	28	30	192x	41.50	16	13.4	7.97	7.81	2.02	2.09

177	C	4	T4	28	30	289x	40.99	16.1	14.7	7.55	7.34	2.79	1.78
178	C	4	T4	28	30	175x	41.54	16	13.3	7.73	7.53	2.60	1.19
179	C	4	T4	28	30	261x	41.49	14.1	13.9	6.60	6.45	2.28	0.92
180	C	4	T4	28	30	269x	42.25	17.6	15.6	7.66	7.45	2.75	1.44
181	C	4	T4	28	30	218x	41.80	16.7	15	7.55	7.38	2.26	0.79
182	C	4	T4	28	30	241x	42.02	18.7	15.6	7.28	7.07	2.90	1.47
183	C	4	T4	28	30	287x	41.61	15.5	13.4	7.42	7.22	2.71	1.51
184	C	4	T4	28	30	150x	41.22	13.1	14	6.39	6.20	2.83	1.57
193	C	5	T5	28	80	200x	41.01	12.7	17	7.34	7.12	3.01	1.01
194	C	5	T5	28	80	287x	41.61	17	14.3	7.88	7.64	3.06	0.94
195	C	5	T5	28	80	269x	42.25	14	15.4	7.45	7.22	3.10	1.20
196	C	5	T5	28	80	241x	42.16	15.8	14.6	7.59	7.37	2.91	0.96
197	C	5	T5	28	80	261x	42.35	14	13.1	6.69	6.59	1.50	1.37
198	C	5	T5	28	80	218x	41.49	17.1	13.7	7.20	6.96	3.35	0.80
199	C	5	T5	28	80	187x	41.58	14.8	12.9	7.10	6.86	3.39	0.89
200	C	5	T5	28	80	175x	41.41	18.3	12.9	7.24	6.95	4.02	1.38
209	C	5	T5	28	80	241x	41.05	16	15.7	7.77	7.51	3.36	1.44
210	C	5	T5	28	80	253x	41.11	16.9	13.7	7.38	7.17	2.86	1.19
211	C	5	T5	28	80	175x	41.16	16.4	14	7.50	7.22	3.75	1.22
212	C	5	T5	28	80	200x	41.32	18.8	15.3	7.25	7.04	2.91	1.41
213	C	5	T5	28	80	285x	41.32	16.7	12.7	7.70	7.52	2.35	1.35
214	C	5	T5	28	80	289x	40.62	17	12.7	8.21	7.94	3.30	1.17
215	C	5	T5	28	80	150x	41.27	16.2	13.2	7.53	7.33	2.67	1.18
216	C	5	T5	28	80	287x	41.61	19.3	14.1	7.73	7.55	2.34	1.02

Table A4: Hen meat quality data

hen	flock	pen	trt	temp	RH	initial pH BP	ult pH BP	initial pH BS	ult pH BS	pH thigh	breast L*	breast a*	breast b*	thigh L*	thigh a*	thigh b*
9	A	1	T1	20	30	6.79	5.86	6.39	5.76	5.65	48.815	4.95	-3.99	48.965	14.765	1.9
10	A	1	T1	20	30	6.44	5.6	6.28	5.61	5.98	50.855	5.64	-2.02	49.505	13.54	0.795
11	A	1	T1	20	30	6.74	5.66	6.48	5.54	5.74	53.535	4.725	-1.115	49.105	13.015	0.685
12	A	1	T1	20	30	6.79	5.88	6.47	5.65	5.96	49.745	5.45	-3.125	50.655	10.265	-0.105
13	A	1	T1	20	30	6.68	5.66	6.58	5.53	5.83	52.91	5.165	-2.765	50.58	13.73	1.22
14	A	1	T1	20	30	6.76	5.71	6.51	5.64	5.96	48.22	6.415	-2.185	48.795	14.76	1.85
15	A	1	T1	20	30	6.31	5.69	6.45	5.59	5.91	49.59	4.975	-3.235	47.525	13.775	-0.51
16	A	1	T1	20	30	6.52	5.62	6.21	5.5	5.58	50.87	5.825	-2.535	55.42	11.41	0.455
25	A	2	T2	20	80	6.34	5.69	6.3	5.67	5.86	52.775	4.425	-2.86	49.555	12.12	-0.53
26	A	2	T2	20	80	6.39	5.61	6.5	5.77	6	50.35	4.615	-3.195	48.9	12.26	-0.645
27	A	2	T2	20	80	6.5	5.71	6.39	5.76	6.04	49.67	4.61	-3.09	51.51	13.44	2.855
28	A	2	T2	20	80	7.02	5.61	6.55	5.64	5.83	52.12	4.635	-2.33	58.66	9.17	2.1
29	A	2	T2	20	80	6.43	5.66	6.41	5.75	5.95	48.27	4.92	-3.925	46.095	15.89	1.035
30	A	2	T2	20	80	6.62	5.67	6.65	5.66	5.98	51.82	5.03	-2.215	51.235	11.165	0.52
31	A	2	T2	20	80	6.62	5.7	6.51	5.52	5.81	52.185	5.915	-1.87	51.265	15.52	2.79
32	A	2	T2	20	80	6.61	5.7	6.46	5.54	5.52	51.265	4.52	-2.9	49.335	18.08	4.36
33	A	3	T3	-18	.	6.93	5.67	6.62	5.61	6.4	48.1	5.07	-3.11	50.255	12.85	-0.02
34	A	3	T3	-18	.	6.77	5.89	6.69	5.82	6.39	47.95	4.435	-3.475	47.985	11.415	-1.85
35	A	3	T3	-18	.	6.44	5.83	6.6	5.8	6.47	49.17	4.55	-3.395	40.305	17.68	-2.11
36	A	3	T3	-18	.	6.76	5.88	6.7	5.8	7.01	50.21	4.33	-3.885	46.42	13.755	-1.98
37	A	3	T3	-18	.	6.54	5.5	6.72	5.88	6.72	45.87	5.025	-4.38	46.86	14.585	-1.315
38	A	3	T3	-18	.	6.7	5.84	6.75	5.88	6.6	45.675	4.895	-4.38	46.88	16.73	0.875
39	A	3	T3	-18	.	6.69	5.96	6.5	5.98	6.32	46.965	5.155	-4.435	45.5	15.485	0.87
40	A	3	T3	-18	.	6.77	5.72	6.64	5.75	6.28	49.04	3.81	-2.495	46.81	15.38	-1.2
89	B	1	T1	20	30	6.74	5.76	6.66	5.7	5.91	51.26	4.59	-2.945	49.07	10.865	-1.125
90	B	1	T1	20	30	6.83	5.81	6.39	5.65	6.26	48.935	4.91	-3.125	55.425	12.8	2.59

91	B	1	T1	20	30	6.7	5.68	6.46	5.7	5.93	51.415	4.66	-3.12	54.15	13.115	1.5
92	B	1	T1	20	30	6.68	5.66	6.44	5.67	5.86	51.53	4.36	-3.36	55.175	8.895	0.405
93	B	1	T1	20	30	6.86	5.71	6.54	5.64	5.88	47.79	5.38	-4.01	50.14	14.325	1.535
94	B	1	T1	20	30	6.66	5.66	6.57	5.63	5.99	52.535	3.48	-3.205	51.525	13.175	2.075
95	B	1	T1	20	30	7	5.7	6.66	5.7	6.1	48.71	4.9	-3.195	54.06	11.79	1.905
96	B	1	T1	20	30	6.97	5.69	6.65	5.64	5.94	52.37	4.24	-2.89	52.345	13.045	0.825
105	B	2	T2	20	80	6.61	5.64	6.57	5.56	5.85	51.57	4.725	-2.43	51.585	15.14	1.275
106	B	2	T2	20	80	6.57	5.62	6.4	5.67	5.76	53.365	3.775	-2.92	51.335	11.51	-3.765
107	B	2	T2	20	80	6.57	5.67	6.56	5.68	5.6	52.23	4.62	-2.445	50.55	15.46	2.13
108	B	2	T2	20	80	6.94	5.62	6.43	5.56	5.78	49.895	4.735	-3.04	50.56	13.58	0.835
109	B	2	T2	20	80	6.46	5.67	6.51	5.59	5.81	50.045	4.825	-4.03	50.755	13.92	0.59
110	B	2	T2	20	80	6.93	5.6	6.79	5.59	5.88	53.26	4.53	-2.975	52.94	14.175	4.19
111	B	2	T2	20	80	6.74	5.65	6.52	5.63	5.89	51.62	5.25	-3.17	50.635	13.945	0.95
112	B	2	T2	20	80	6.74	5.58	6.53	5.64	5.79	51.775	4.505	-2.86	49.425	13.9	-3.01
121	B	3	T3	-18	.	6.87	5.64	6.77	5.61	6.37	47.925	5.295	-3.145	53.1	14.31	2.265
122	B	3	T3	-18	.	6.39	5.64	6.7	5.61	5.99	48.155	3.84	-4.295	49.92	14.89	1.455
123	B	3	T3	-18	.	6.45	5.73	6.7	5.62	6.59	49.405	4.075	-4.885	48.75	15.065	1
124	B	3	T3	-18	.	6.41	5.74	6.79	5.65	6.13	50.33	4.08	-4.355	48.635	13.9	1.24
125	B	3	T3	-18	.	6.51	5.65	6.8	5.63	6.45	48.44	5.505	-3.82	49.75	15.645	2.96
126	B	3	T3	-18	.	6.7	5.77	6.85	5.62	6.32	49.465	3.78	-3.95	47.165	16.62	1.67
127	B	3	T3	-18	.	6.62	5.63	6.54	5.64	6.36	48.695	4.87	-4.36	48.96	14.505	0.64
128	B	3	T3	-18	.	6.59	5.74	6.77	5.64	6.13	47.195	4.48	-4.27	49.98	14.99	-1.09
185	C	1	T1	20	30	6.68	5.74	6.63	5.72	6.11	48.715	5.545	-4.005	51.145	17.08	2.69
186	C	1	T1	20	30	6.75	5.72	6.77	5.6	5.92	53.145	4.36	-2.465	50.165	12.945	0.065
187	C	1	T1	20	30	6.85	5.67	6.54	5.6	5.93	51.045	4.55	-3.215	52.95	16.83	4.48
188	C	1	T1	20	30	6.87	5.74	6.5	5.66	5.97	53.21	3.94	-2.89	50.57	12.82	-0.335
189	C	1	T1	20	30	6.67	5.7	6.37	5.63	5.84	53.5	5.165	-3.045	54.395	12.375	-0.575
190	C	1	T1	20	30	6.96	5.74	6.61	5.69	5.91	51.25	5.015	-3.025	45.675	12.705	-2.63
191	C	1	T1	20	30	6.8	5.75	6.62	5.72	6.11	52.655	4.215	-3.225	49.685	12.165	-0.715
192	C	1	T1	20	30	6.57	5.7	6.77	5.62	5.93	53.28	4.9	-1.735	53.36	14.935	2.555

201	C	2	T2	20	80	6.81	5.75	6.85	5.63	6.01	50.57	4.255	-3.58	42.59	12.04	-0.705
202	C	2	T2	20	80	6.49	5.75	6.71	5.64	6.11	49.625	5.045	-3.415	46.96	16.225	1.805
203	C	2	T2	20	80	6.34	5.66	6.7	5.61	5.92	53.985	4.19	-2.41	55.72	10.955	0.245
204	C	2	T2	20	80	6.81	5.87	6.59	5.74	6.32	51.68	5.355	-2.84	53.095	13.595	1
205	C	2	T2	20	80	6.8	5.74	6.73	5.7	6.09	50.185	5.505	-3.255	53.715	12.45	1.35
206	C	2	T2	20	80	6.88	5.81	6.6	5.67	5.99	49.135	5.7	-3.525	52.91	13.23	1.265
207	C	2	T2	20	80	6.83	5.72	6.68	5.67	6.04	52.185	5.215	-2.34	53.145	13.355	0.6
208	C	2	T2	20	80	6.81	5.9	6.59	5.59	6.12	51.38	4.365	-3.525	52.055	12.55	-0.045
217	C	3	T3	-18	.	6.51	5.69	6.8	5.77	6.43	50.91	4.735	-4.125	49.6	13.76	0.3
218	C	3	T3	-18	.	6.88	5.91	6.85	5.71	6.15	49.695	4.485	-2.885	48.63	15.56	0.185
219	C	3	T3	-18	.	6.86	5.88	6.75	5.67	6.25	51.115	4.81	-3.105	53.48	13.1	0.745
220	C	3	T3	-18	.	6.57	5.92	6.94	5.85	6.07	48.595	5.445	-4.57	44.55	19.865	1.925
221	C	3	T3	-18	.	6.83	5.75	6.65	5.67	6.15	52.715	3.915	-3.465	48.845	12.965	-3.71
222	C	3	T3	-18	.	6.76	5.81	6.68	5.76	6.62	51.215	5.14	-2.9	52.415	12.48	-0.405
223	C	3	T3	-18	.	6.7	5.81	6.68	5.75	6.53	50.92	3.635	-3.255	48.57	15.27	0.73
224	C	3	T3	-18	.	6.74	5.88	6.72	5.78	6.53	49.49	4.3	-4.19	47.88	17.735	-1.355
81	B	4	T4	28	30	6.83	5.72	6.39	5.65	5.78	49.085	4.56	-4.12	51.43	15.065	0.93
82	B	4	T4	28	30	6.24	5.67	6.58	5.6	5.88	51.065	4.83	-2.9	51.365	13.2	0.885
83	B	4	T4	28	30	6.72	5.74	6.13	5.64	5.85	48.69	4.38	-4.115	51.325	12.045	-0.59
84	B	4	T4	28	30	6.88	5.69	6.38	5.64	6.01	50.885	3.945	-2.815	48.21	13.495	0.675
85	B	4	T4	28	30	6.66	5.62	6.45	5.58	5.93	51.72	5.64	-3.44	49.3	15.005	2.365
86	B	4	T4	28	30	6.54	5.75	6.33	.	5.98	49.13	5.41	-3.54	52.065	15.62	1.075
87	B	4	T4	28	30	6.53	5.72	6.4	5.64	5.95	46.625	5.855	-3.885	51.965	12.175	-3.115
88	B	4	T4	28	30	6.78	5.67	6.43	5.61	5.98	55.335	3.785	-2.445	48.31	16.68	1.665
97	B	5	T5	28	80	6.64	5.63	6.49	5.6	5.88	51.91	4.28	-3.04	50.815	17.045	2.43
98	B	5	T5	28	80	6.55	5.59	6.63	5.6	5.74	55.045	4.625	-2.205	55.68	13.035	2.675
99	B	5	T5	28	80	6.78	5.63	6.68	5.59	5.88	50.02	5.87	-3.32	54.21	12.005	-0.855
100	B	5	T5	28	80	6.58	5.65	6.42	5.62	5.8	55.1	4.915	-6.65	52.885	16.125	0.375
101	B	5	T5	28	80	6.58	5.62	6.51	5.62	5.86	52.345	3.945	-2.5	51.58	10.18	-1.34
102	B	5	T5	28	80	6.66	5.62	6.57	5.64	5.82	52.52	4.61	-2.135	51.335	14.89	0.975

103	B	5	T5	28	80	6.54	5.63	6.62	5.62	5.64	52.32	4.94	-2.46	50.71	11.995	-1.375
104	B	5	T5	28	80	6.8	5.63	6.6	5.63	5.85	51.95	5.195	-3.225	49.415	15.485	1.115
113	B	4	T4	28	30	6.67	5.63	6.4	5.6	5.92	50.71	4.845	-3.815	55.25	13.115	3.685
114	B	4	T4	28	30	6.65	5.61	6.48	5.59	5.93	50.305	5.59	-2.685	49.99	14.865	-0.21
115	B	4	T4	28	30	6.8	5.63	6.74	5.61	5.91	51.885	4.895	-4.38	49.795	15.59	1.855
116	B	4	T4	28	30	6.66	5.63	6.65	5.56	5.86	52.93	4.07	-2.935	48.51	13.485	-0.295
117	B	4	T4	28	30	6.87	5.57	6.67	5.61	5.89	52.615	5.15	-1.76	51.485	11.985	-0.02
118	B	4	T4	28	30	6.46	5.63	6.51	5.56	6.04	51.095	4.63	-2.625	51.74	15.595	0.535
119	B	4	T4	28	30	6.63	5.61	6.69	5.56	5.85	55.88	4.355	-1.74	45.61	17.125	-0.585
120	B	4	T4	28	30	6.63	5.64	6.64	5.6	5.8	51.905	4.28	-3.18	49.075	19.525	2.475
177	C	4	T4	28	30	6.55	5.71	6.73	5.64	6.21	49.655	5.695	-3.255	51.31	14.59	1.445
178	C	4	T4	28	30	6.68	5.79	6.77	5.78	6	48.285	5.765	-4.84	47.475	12.85	-2.755
179	C	4	T4	28	30	6.82	5.75	6.68	5.73	6.42	50.29	4.825	-3.995	50.74	15.88	0.955
180	C	4	T4	28	30	6.57	5.75	6.67	5.72	6.01	51	4.835	-3.54	50.275	16.245	3.96
181	C	4	T4	28	30	6.87	5.81	6.67	5.76	6.03	48.29	4.725	-4.93	53.135	11.835	0.505
182	C	4	T4	28	30	6.85	5.85	6.85	5.84	6.25	49.61	4.575	-3.61	48.54	12.535	-2.245
183	C	4	T4	28	30	6.37	5.81	6.65	5.72	6.08	50.13	5.89	-3.475	46.945	20.405	2.885
184	C	4	T4	28	30	6.6	5.72	6.38	5.63	5.99	51.845	5.195	-3.72	49.77	12.205	-2.6
193	C	5	T5	28	80	6.84	5.81	6.74	5.74	6.08	50.51	4.7	-3.45	49.245	14.03	0.27
194	C	5	T5	28	80	6.72	5.73	6.54	5.65	5.93	50.41	5.425	-3.35	51.32	15.94	1.92
195	C	5	T5	28	80	6.63	5.74	6.61	5.67	5.93	52.47	5.42	-2.63	48.135	14.025	0.68
196	C	5	T5	28	80	6.87	5.74	6.82	5.68	5.92	52.135	4.725	-2.88	53.335	14.585	1.535
197	C	5	T5	28	80	6.76	5.84	6.81	5.65	6.14	47.295	4.695	-4.665	47.825	12.94	-0.92
198	C	5	T5	28	80	6.62	5.72	6.73	5.62	5.91	53.98	5.545	-2.66	47.985	17.02	1.215
199	C	5	T5	28	80	6.32	5.78	6.58	5.71	5.92	55.26	3.58	-3.3	54.74	12.36	1.465
200	C	5	T5	28	80	6.49	5.74	6.51	5.62	5.89	52.605	3.97	-3.21	52.58	12.76	0.175
209	C	5	T5	28	80	6.74	5.73	6.76	5.67	5.99	51.235	5.395	-4.055	48.775	10.4	-1.655
210	C	5	T5	28	80	6.74	5.71	6.76	5.59	6.08	53.515	4.875	-3.13	45.47	18.905	0.755
211	C	5	T5	28	80	6.89	5.68	6.88	5.63	6.11	52.825	4.4	-2.82	50.88	12.455	-2.73
212	C	5	T5	28	80	6.82	5.73	6.76	5.65	6.02	53.845	4.245	-2.215	48.21	13.135	-2.11

213	C	5	T5	28	80	6.76	5.68	6.84	5.63	6.02	53.74	4.01	-2.33	49.63	12.565	0.74
214	C	5	T5	28	80	6.66	5.7	6.76	5.57	5.97	53.48	4.185	-1.975	49.14	17.535	1.19
215	C	5	T5	28	80	6.62	5.72	6.66	5.57	6.04	51.1	4.24	-2.815	49.025	10.54	-0.76
216	C	5	T5	28	80	6.79	5.67	6.65	5.59	5.91	54.44	4.415	-2.37	48.465	16.095	0.42

Appendix B: Time Budgets during the last 4h of simulated transport

Table B1: Time budgets for toms exposed to -18°C

Trt	-18C															
Sex	M															
				Stand			**Sit/Lay**									
Flock #	Crate #		No Obsv	Activity	Shiver	Ptiloerect	Rest/Still	Activity	Vocalize	Survey	Peck	Preen	Stretch	Shiver	Huddle	Ptiloerect
1	7		0	1	0	5	0	2	0	0	0	4	1	6	115	62
	8		0	1	0	0	0	2	1	1	1	2	0	3	111	74
2	5		0	1	1	2	0	1	0	0	0	12	0	5	49	125
	8		0	0	0	0	0	1	0	2	1	28	0	3	69	92
3	7		0	3	0	0	4	3	0	0	1	6	0	3	32	144
	8		0	1	0	0	4	3	1	3	0	8	0	6	10	160
		% time	0	0.595238	0.085034	0.595238	0.680272	1.020408	0.170068	0.510204	0.255102	5.102041	0.085034	2.210884	32.82313	55.86735

Table B2: Time budgets for toms exposed to 20°C 30% RH

Trt	20C 30RH															
Sex	M															
				Stand				**Sit/Lay**							**Panting**	
Flock #	Crate #		No Obsv	Still	Activity	Peck	Preen	Rest/Still	Activity	Survey	Peck	Preen	Stretch	Head-rest	Stand	Sit/lay
1	7		56	5	6	2	0	70	0	0	1	0	0	8	2	46
	8		57	2	4	1	0	80	2	0	0	0	0	19	0	31
2	7		0	2	2	0	0	150	12	1	1	3	1	14	0	10
	8		0	0	1	0	0	137	3	1	0	1	0	8	0	45
3	5		10	0	4	0	1	165	5	3	1	0	0	0	1	6
	6		41	0	6	0	0	116	6	11	1	1	0	3	2	9
		% time	13.94558	0.765306	1.955782	0.255102	0.085034	61.05442	2.380952	1.360544	0.340136	0.42517	0.085034	4.421769	0.42517	12.5

Table B3: Time budgets for toms exposed to 20°C 80% RH

Trt	20C 80RH																			
Sex	M																			
				Stand		**Sit/Lay**							**Panting**							
Flock #	Crate #		No Obsv	Still	Activity	Rest/Still	Activity	Survey	Peck	Preen	Wing-drop	Head-rest	Stand	Sit/lay	Activity	Survey	Stretch	Vocalize	Head-rest	Exposing s
1	5		8	1	0	81	5	0	0	0	1	78	0	22	0	0	0	0	0	0
	7		0	1	2	110	2	2	0	1	0	56	1	21	0	0	0	0	0	0
2	7		49	0	3	101	2	7	0	0	0	4	0	9	0	0	0	0	20	1
	8		26	2	1	124	1	2	3	1	0	4	0	18	0	0	0	0	14	0
3	7		10	0	0	31	0	0	0	1	0	89	0	42	5	1	0	4	13	0
	8		0	3	1	25	0	0	2	1	0	36	2	111	6	4	1	1	3	0
		% time	7.908163	0.595238	0.595238	40.13605	0.85034	0.935374	0.42517	0.340136	0.085034	22.70408	0.255102	18.96259	0.935374	0.42517	0.085034	0.42517	4.251701	0.085034

Table B4: Time budgets for toms exposed to 28°C 30% RH

Trt	28C 30RH																
Sex	M																
				Stand			**Sit/Lay**						**Panting**				
Flock #	Crate #		No Obsv	Still	Activity	Head-rest	Rest/Still	Activity	Survey	Vocalize	Peck	Head-rest	Stand	Sit/lay	Activity	Survey	Head-rest
2	5		42	0	3	0	68	2	0	0	1	0	0	70	0	0	10
	6		59	1	0	0	77	2	0	0	0	0	0	45	0	1	11
2	6		61	3	5	0	19	3	1	0	2	5	0	76	0	0	21
	7		75	0	1	0	16	1	0	0	0	4	0	72	0	0	27
3	7		0	3	3	3	29	3	3	1	0	24	12	29	4	4	78
	8		1	0	0	0	38	4	4	0	0	37	4	37	3	3	65
		% time	20.2381	0.595238	1.020408	0.255102	21.0034	1.27551	0.680272	0.085034	0.255102	5.952381	1.360544	27.97619	0.595238	0.680272	18.02721

Table B5: Time budgets for toms exposed to 28°C 80% RH

Trt	28C 80RH																		
Sex	M																		
				Stand		**Sit/Lay**					**Panting**								
Flock #	Crate #		No Obsv	Still	Activity	Rest/Still	Activity	Peck	Preen	Head-rest	Stand	Sit/lay	Activity	Survey	Stretch	Vocalize	WingDroop	Head-rest	ExposeSkin
2	5		54	1	0	6	0	1	0	10	5	72	13	0	1	0	4	21	8
	6		80	0	1	8	1	0	0	13	9	53	4	1	0	0	0	19	7
3	5		1	0	0	11	0	0	0	6	23	27	7	1	0	5	5	68	42
	6		0	0	0	5	0	0	0	6	0	23	1	7	0	0	0	93	61
3	5		13	0	0	22	2	0	1	13	0	98	5	2	0	1	0	19	20
	6		34	0	0	29	0	0	0	5	0	71	2	1	0	1	0	45	8
		% time	15.47619	0.085034	0.085034	6.887755	0.255102	0.085034	0.085034	4.506803	3.146259	29.2517	2.721088	1.020408	0.085034	0.595238	0.7653061	22.53401	12.414966

Table B6: Time budgets for hens exposed to -18°C

Trt	-18C																
Sex	F																
				Stand							**Sit/Lay**						
Flock #	Crate #		No Obsv	Still	Activity	Survey	Peck	Preen	Shiver	Ptiloerect	Activity	Vocalize	Peck	Preen	Shiver	Huddle	Ptiloerect
1	1		1	0	4	1	1	5	7	47	5	0	0	6	5	77	37
	2		0	0	6	0	0	2	1	7	2	0	2	6	2	155	13
2	3		12	0	4	0	1	3	8	33	5	1	0	1	8	77	43
	4		12	0	5	0	0	1	3	4	3	0	0	9	5	75	79
3	1		0	1	8	0	0	4	10	15	5	0	2	3	9	111	28
	4		0	0	4	0	1	1	3	1	2	0	1	9	8	147	19
		% time	2.12585	0.085034	2.636054	0.085034	0.255102	1.360544	2.721088	9.098639	1.870748	0.085034	0.42517	2.891156	3.146259	54.59184	18.62245

Table B7: Time budgets for hens exposed to 20°C 30% RH

Trt	20C 30RH															
Sex	F															
				Stand					**Sit/Lay**							**Panting**
Flock #	Crate #		No Obsv	Still	Activity	Survey	Peck	Preen	Rest/Still	Activity	Vocalize	Survey	Peck	Preen	Head-rest	Sit/lay
1	3		0	6	9	1	3	0	168	1	0	2	4	2	0	0
	4		0	3	7	1	1	0	173	5	0	3	1	2	0	0
2	3		0	6	8	0	1	0	174	4	0	1	1	0	1	0
	4		0	3	7	0	1	0	172	6	2	0	4	0	0	1
3	1		0	2	5	0	1	0	182	3	0	0	1	2	0	0
	4		0	7	2	1	1	3	165	3	0	3	5	6	0	0
		% time	0	2.295918	3.231293	0.255102	0.680272	0.255102	87.92517	1.870748	0.170068	0.765306	1.360544	1.020408	0.085034	0.085034

Table B8: Time budgets for hens exposed to 20°C 80% RH

Trt	20C 80RH														
Sex	F														
				Stand					**Sit/Lay**						**Panting**
Flock #	Crate #		No Obsv	Still	Activity	Survey	Peck	Preen	Rest/Still	Activity	Vocalize	Peck	Preen	Head-rest	Sit/lay
1	3		0	6	10	0	5	2	152	10	0	3	5	0	3
	4		0	8	9	1	0	0	168	5	0	1	2	0	2
2	3		0	9	8	1	0	0	72	5	0	1	0	0	0
	4		2	11	3	0	0	0	77	0	1	1	0	0	1
3	2		0	6	1	2	2	0	167	10	1	3	2	1	1
	3		0	7	4	0	0	1	174	3	0	4	3	0	0
		% time	0.204918	4.815574	3.586066	0.409836	0.717213	0.307377	82.9918	3.381148	0.204918	1.331967	1.229508	0.102459	0.717213

Table B9: Time budgets for hens exposed to 28°C 30% RH

Trt	28C 30RH																	
Sex	F																	
				Stand						**Sit/Lay**							**Panting**	
Flock #	Crate #		No Obsv	Still	Activity	Vocalize	Survey	Peck	Preen	Rest/Still	Activity	Vocalize	Survey	Peck	Preen	Head-rest	Stand	Sit/lay
2	1		0	2	3	0	0	0	0	185	1	0	0	1	0	0	0	4
	2		0	7	8	0	0	0	1	162	6	1	0	2	0	2	0	7
2	1		0	10	1	1	0	3	1	168	2	2	1	0	0	2	1	4
	2		4	14	8	4	2	1	0	154	0	0	1	1	2	0	1	4
3	2		0	6	10	0	0	1	1	160	3	0	0	0	1	0	4	10
	3		0	8	8	0	0	0	0	156	5	0	0	1	1	0	3	14
		% time	0.340136	3.996599	3.231293	0.42517	0.170068	0.42517	0.255102	83.7585	1.445578	0.255102	0.170068	0.42517	0.340136	0.340136	0.765306	3.656463

Table B10: Time budgets for hens exposed to 28°C 80% RH

Trt	28C 80RH																			
Sex	F																			
				Stand						**Sit/Lay**							**Panting**			
Flock #	Crate #		No Obsv	Still	Activity	Vocalize	Survey	Peck	Preen	Rest/Still	Activity	Vocalize	Survey	Peck	Preen	Head-rest	Stand	Sit/lay	Activity	Vocalize
2	1		20	13	6	2	0	2	0	53	4	1	0	0	0	13	10	71	1	0
	2		13	4	2	0	0	0	0	62	3	3	0	0	0	17	9	80	2	1
3	1		0	10	8	0	1	0	0	116	7	0	0	2	2	22	1	27	0	0
	4		0	9	2	0	0	0	0	128	3	0	2	5	2	6	1	38	0	0
3	2		0	7	8	0	0	1	1	109	5	0	0	1	0	0	8	56	0	0
	3		0	6	1	0	0	0	0	114	8	0	0	3	2	0	10	52	0	0
		% time	2.806122	4.166667	2.295918	0.170068	0.085034	0.255102	0.085034	49.4898	2.55102	0.340136	0.170068	0.935374	0.510204	4.931973	3.316327	27.55102	0.255102	0.085034