Evaluation of infrared technology for predicting transport stress and meat quality in market pigs

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Overall Abstract

Three experiments were conducted to evaluate infrared technology as a tool for predicting transport stress and pale soft and exudative (PSE) meat traits in market pigs. Experiment 1 compared the ability of two digital infrared thermographic cameras [research grade (RG) and consumer grade (CG)] to detect temperature change in market pigs (N=168 market pigs, BW=111.5±13.2 kg). There were two treatment groups: Control and Handling treatment (N=84 per treatment). Control pigs remained in their home pen while Handling treatment pigs received a mild handling stressor (walking a distance of approximately 100m). The ocular (OT) and body temperature (BT) of all pigs were measured using both cameras at two time points: before (baseline) and approximately 1 hr later (after Handling treatment pigs were moved). Experiment 2 compared pre- and post-transport ocular and body temperatures of market pigs to determine if pre-transport temperatures were predictive of post-transport temperatures using CG digital infrared cameras. In Experiment 2, pigs (N=120, BW=105.1 ± 4.9 kg) were transported in five replicates (20-25 pigs/replicate) for ~2 h to an abattoir during summer. Thermographic ocular and body images were collected from each pig at three time points; twice before and once after transport (T1: three days prior to transport, T2: one day before transport, and T3: in lairage post-transport). Experiment 3 was conducted using animals from Experiment 2. The objective of Experiment 3 was to determine if infrared technology can predict meat quality based on the post-transport ocular and body temperatures (T3) collected at the packing plant prior to slaughter. At slaughter, blood samples were collected for cortisol, glucose and lactate analyses. Carcass pH was taken at 1 and 3 h postmortem and loin samples were collected for meat quality (ultimate pH, meat color, drip loss and meat tenderness) assessment. Data collected in Experiment 1 were analyzed using Pearson correlations, linear regression and a mixed model with main effects: treatment, time and their interaction, with pen as a random factor using SAS (SAS 9.4). Data collected in Experiment 2 were analyzed using Pearson correlations and regression analysis, while data collected in Experiment 3 were analyzed using Pearson correlations, linear regression and mixed model analyses in SAS. In Experiment 1, the infrared measures from RG and CG cameras were positively ($r=0.93$, $P<0.05$) correlated. In addition, handling treatment led to increases ($P<0.05$) in body and

ocular temperatures in handled pigs compared to unhandled controls. In Experiment 2, significant positive correlations were found between T1 and T2 body temperatures**.** Moreover, the regression analyses showed strong associations (r^2=0.80, P=0.01) between T1 and T2 body temperatures. Correlations between T1 and T2 ocular temperatures were non-significant, and there were no relationships between T1 and T3, or T2 and T3 temperatures for body or ocular measures. Experiment 3 showed positive correlations (r=0.40, P<0.010) between IR ocular temperatures post-transport and blood cortisol at slaughter, suggesting a relationship between temperature and stress physiology in pigs. Meat yellowness (*b**) increased with elevated body temperatures (r=0.2, P <0.001). Meat tenderness increased with increase in IR ocular and body temperatures post-transport (r=-0.51, P<0.001). Pigs with high IR body temperatures post-transport/pre-slaughter had poorer meat quality characterized by pale soft and exudative (PSE), moderately pale soft and exudative (MPSE), pale firm and normal (PFN) carcasses postmortem. In conclusion, no correlation was found between on-farm and post-transport IR temperatures. However, IR body temperatures post-transport were predictive of meat quality traits in market pigs. Results in this thesis support the potential for infrared technology to identify stressed or febrile pigs on-farm and to predict pork quality before slaughter. Automation of infrared technology in commercial barns or packing plants could allow real-time data collection and monitoring of pig health for improved animal welfare and meat quality.

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List of Abbreviations

RG	research grade
CG	consumer grade
ANOVA	analysis of variance
SAS	statistical analysis software
PSE	pale soft exudative
MPSE	moderately pale soft exudative
PFN	pale firm and normal
RSE	red soft and exudative
DFD	dark firm and dry
ADG	average daily gain
ADFI	average daily feed intake
G:F	gain to feed ratio
RFD	radio frequency digital
MB	mercury bulb
AC	alternate current
DC	direct current
AmbT	ambient temperature
GTT	gastrointestinal temperature
pHu	ultimate pH
LM	longissimus muscle
HPA	hypothalamo-pituitary-axis
PVN	paraventricle nuclei
ACTH	adenocorticotropic hormone
DNA	deoxyribonucleic acid
GWAS	Genome-wide association studies
IR	infrared
IRT	infrared thermography
WBS	Warner-Bratzler shear

WHC	water holding capacity

1.0 Chapter 1. Literature review: body temperature, heat stress in pigs, and application of infrared technology in livestock production

1.1 Introduction

Swine production is faced with multiple problems which undermine the quality and quantity of pork. Variation in pork quality is directly related to physiological changes in pigs perimortem (Correa et al. 2013; Weschenfelder et al. 2013). Several studies have examined the effects of heat stress on pork quality (Schaefer et al. 2012). Most of these physiological and metabolic studies were accomplished using invasive techniques like a rectal thermometer (Stewart et al. 2008) which are labor intensive and unreliable (Soerensen and Pedersen 2015). Market pigs for slaughter must be handled in accordance with the Code of Practice for the Care and Handling of Pigs (National Farm Animal Care Council 2014) which recommends that febrile pigs should be identified and excluded from transport to prevent in-transit deaths, ambulatory and non-ambulatory injuries on arrival at the plant. In addition, there are significant associations between meat pH, thermal stress and pale soft and exudative (PSE) meat traits (Weschenfelder et al. 2013). Pale soft and exudative meats are characterized by poor carcass yield, high cooking loss, and low juiciness (Honkavaara 1988) which leads to significant economic loss (Edwards et al. 2010).

The swine industry is in need of safe and non-invasive methods for screening market pigs prior to transport. Currently, there is limited information on the methods for identifying individuals or groups of pigs, which are likely to yield poor carcasses at slaughter. Cook et al. (2015) reported that digital thermography could be helpful in detecting temperature change in animals for disease diagnoses.

Infrared thermography (IRT) is a non-invasive technique, which allows a remote sensing of animal's body temperatures (Warriss et al. 2006). Infrared thermography converts infrared radiation emitted by a heat source into pixel intensity thereby providing a thermogram of the

measured surface (Griffith et al. 2002). Infrared thermography is a more reliable temperature measuring tool in animals than the invasive techniques (Loughmiller et al. 2001). Its use for measuring temperature in animals reduces the risk of contacting and spreading infections in a herd (Ludwig et al. 2007). Hence, thermographic imaging could be useful for predicting disease and meat quality in market pigs, and there is need for further studies to identify the best region for IRT imaging in pigs for improved animal welfare.

1.1.1 Body temperature and thermoregulation in pigs

The internal temperature of an animal is referred to as its body temperature (Juxiong and Yang 2011). This temperature serves as a reference when estimating the physiological and health status of the animal. A change in body temperature could be attributed to a deviation in the health and function of animals. Changes in body temperature can result from disease, stress, metabolic disorders or other external factors (Yufeng and Yanping 2012). Pig mortality could be prevented by early detection and treatment of febrile pigs on-farm (Lu et al. 2015). Therefore, body temperatures are very helpful in disease diagnoses, detection of severity and treatment. Early detection of infectious diseases in groups of pigs could control the early epidemics and reduce significant economic loss.

The average skin surface temperature of pigs under thermoneutral conditions is 38.3°C (Soerensen and Pedersen 2015). In resting piglets, internal body temperature is around 39.3°C, 38.8 °C in gilts and 38.3°C in multiparous sows (Soerensen and Pedersen 2015). Temperature regulation is essential for the maintenance of homeostasis, a condition needed for optimum performance. Animals modify their behaviours as a natural mechanism of regulating their body temperature. They adjust their location and posture within the environment to either absorb or evolve heat

(Huynh et al. 2005b). Examples of such behaviours include staying under the shade during hot days, or huddling with other pigs in cold weather.

Thermoregulation in homeotherms ensures that metabolic heat is exchanged within the body through cellular and vascular membranes, and between a body and its environment (Taylor et al. 2014). The regulation of core body temperature, amidst the prevailing environmental conditions is achieved by some behavioural and autonomic mechanisms. The preoptic area of the hypothalamus plays an important role in coordinating autonomic thermo-effectors for body heat exchange (Almeida et al. 2015). However, the mechanisms and pathways of the nervous systems responsible for activating behavioural thermoregulation in animals are unclear. The skin, which represents the interface between the body and the environment, plays a significant role in evoking behavioural thermoregulatory mechanisms (Almeida et al. 2015). Thermoregulatory behaviours are essential in helping the body adjust to high and low environmental temperatures (Almeida et al. 2015). This mechanism involves continuous and variable metabolic heat production and convective and conductive heat transport through tissues (Taylor et al. 2014**)**. Under high environmental temperature, heat is lost from the body through radiation, conduction, convection and evaporation (Curtis 1983). In other animal species like horses, monkeys, and apes, heat is lost through the evaporation of water and sweat under high environmental temperatures. This is achieved through the activation of skin's cutaneous vasodilatation causing a reduction of core-to-shell and shell-to-ambient thermal gradients (Almeida et al. 2015; Taylor et al. 2014**).** Unlike these species, pigs do not sweat and require behavioral adjustments to cope under elevated environmental temperatures.

1.1.2 Thermoneutral zone in market pigs

The thermoneutral zone for finishing pigs is between 10 and 23.9°C (Myer and Bucklin 2001). Temperatures above 23.9°C led to a reduction in feed intake and growth performance in pigs (Kouba et al. 2001). The decrease in feed intake at high temperatures (eg. >23.9°C) is a response to lower the metabolic heat produced during feed intake (Collin et al. 2001).

White et al. (2008) studied the influence of ambient conditions (23.9°C or 32.2°C) and space (0.66 or 0.9365 m^2 per pig) on growth and carcass lipid firmness in grow-finish gilts. The pigs were assigned to ambient conditions either within (23.9°C) or above (32.2°C) their zone of thermal comfort. Pigs housed at 32.2°C compared with 23.9°C had reduced ADG and ADFI, reduced G:F ratios, and altered carcass quality. Providing more pen space per pig resulted in increased ADG and ADFI in both 23.9°C and 32.2°C ambient conditions. These reports corroborated with the previous observations that housing temperatures above optimum levels for growing pigs decrease feed intake and growth rate (Myer and Bucklin 2001).

1.1.3 Infrared thermography

Infrared digital cameras detect variations in body temperature (Bertoni et al.2020) that are caused by metabolic changes in pigs (Mota-Rojas et al.2020). The IRT concept was introduced as a form of disease surveillance without the need to restrain animals. Most commonly, IRT are been used in airport screening to detect diseases (Bitar 2009) but has also been applied to veterinary diagnostics (McCafferty 2007). The IRT has mainly been used in veterinary medicine to detect localized infections or inflammations, such as mastitis, foot and mouth disease, or hypertemia (figure 1) in animals (Rainwater-Lovett et al.2009). Infrared thermography evaluates all conditions that are connected with blood flow such as vasodilatation, hyperthermia, hyperperfusion,

hypermetabolism, hypervascularization and hyperaemia (Bagavathiappan et al. 2009).

Infrared thermography has been deployed for evaluating of animals' responses to stress (Casas-Alvarado et al. 2020; Mota-Rojas et al. 2020). Infrared technology has been used in several studies to evaluate stress in pigs, including screening of body (Warriss et al. 2006) the ocular region, and the area behind the ear (Schmidt et al. 2013) to identify pigs under stress. However, Weschenfelder et al. (2013) and Caldara et al. (2014) reported the body/skin as a reliable site for measuring physiological state of pigs under stressful conditions. Rocha et al. (2019) confirmed that IRT could indicate stressful condition in pigs by assessing temperature variations at one or two anatomical locations (back or rump) or at other different body parts. Rocha et al. (2019) measured the temperature responses of 120 pigs on the neck, rump, ocular region and the ear area under gentle handling, and rough handling with exposure to stress. The study examined effect of short (30 min) and long (90 min) transport durations on the salivary cortisol concentrations, rectal temperature, heart rate and behaviour of market pigs. Rocha et al. (2019) reported that the ocular and ear regions showed temperature variations among pigs and recommended these sites as the best infrared thermographic sampling regions in pigs. Moreover, Stewart et al. (2008) and Schaefer et al. (2004) reported that these sites contain abundant capillaries innervated by the sympathetic system that quickly respond with changes in blood flow during stressful situations. Rocha et al. (2019) validated IRT for stress monitoring in pigs based on the observed positive correlations between heart rate and salivary cortisol concentrations with the thermograms from the orbital and ear regions. Furthermore, Ludwig (2013) suggested that these regions could be used for monitoring temperature change in the body because they emit heat quickly due to their low hair density and thin fat coverage.

Factors to consider for valid thermographic imaging include:

(a) Presence of moisture at the region of interest. Banhazi et al. (2009) stated that moisture alters the accuracy of thermographic information.

(b) Image sensitivity. Thermograms of large groups of animals could lower sensitivity. A temperature change in one individual animal could be masked by the temperature of the herd. Cook et al. (2015) observed that the spatial distribution of pigs in group images affected the temperature recorded. According to Escobar et al. (2007), only one animal in a group needs to show a temperature variation for thermographic camera to detect the variation.

(c) Solar radiation and wind speed. Church et al. (2014) reported that a change in ambient temperature/solar radiation could alter thermographic information of a body. Church et al. (2014) observed that wind speed decreased the eye IRT temperature of pigs by 0.43±0.13°C at approximately 7 km/h and 0.78±0.33°C at higher wind speeds (~12 km/h). In addition, Church et al. (2014) reported that IRT ocular temperature increased by 0.56±0.36°C with increase in solar radiation.

1.1.4 Heat stress in pigs

Heat stress in pigs alters nutrient intake, energy partitioning, and metabolism which depresses growth rate, and reproduction (Baumgard and Rhoads 2013). The effect of heat stress on carcass yield is dependent on the severity of the stress, animal genetics, age, and productive stage of the animal (Tarrant 1989). Cruzen et al. (2017) reported that a short duration of heat stress could have a tremendous negative effect on muscle protein, impairing muscle structure, function, growth, and product quality.

In finisher pigs, heat stress during summer has been associated with increased mortality, weight

loss, low carcass yield, and increased PSE meat traits (>32%) in pigs compared to winter conditions (Guardia et al. 2004). Large White pigs kept in hot weather conditions (27.9 °C and 81% RH) from 35 to 94 kg had reduced feed intake (−13%) and average daily gain (−12%), higher pHu of longissimus dorsi (5.71 vs 5.52), lower drip loss (20.7 vs 21.4%) and fat content than those reared in thermoneutral conditions (20 °C and 75% RH) (Rinaldo and Mourot 2001). Finisher pigs exposed to 32.2 °C for 35 days had lower final BW, ADG and feed efficiency with increased bacon lean percentage and higher lean:fat ratio in bacon slices, in contrast to pigs kept at 23.9 °C (White et al. 2008). Under the same feeding regimen, growing pigs exposed to high ambient temperature (31°C) had greater subcutaneous fat than those reared at lower ambient temperature (20°C) (Kouba et al. 2001).

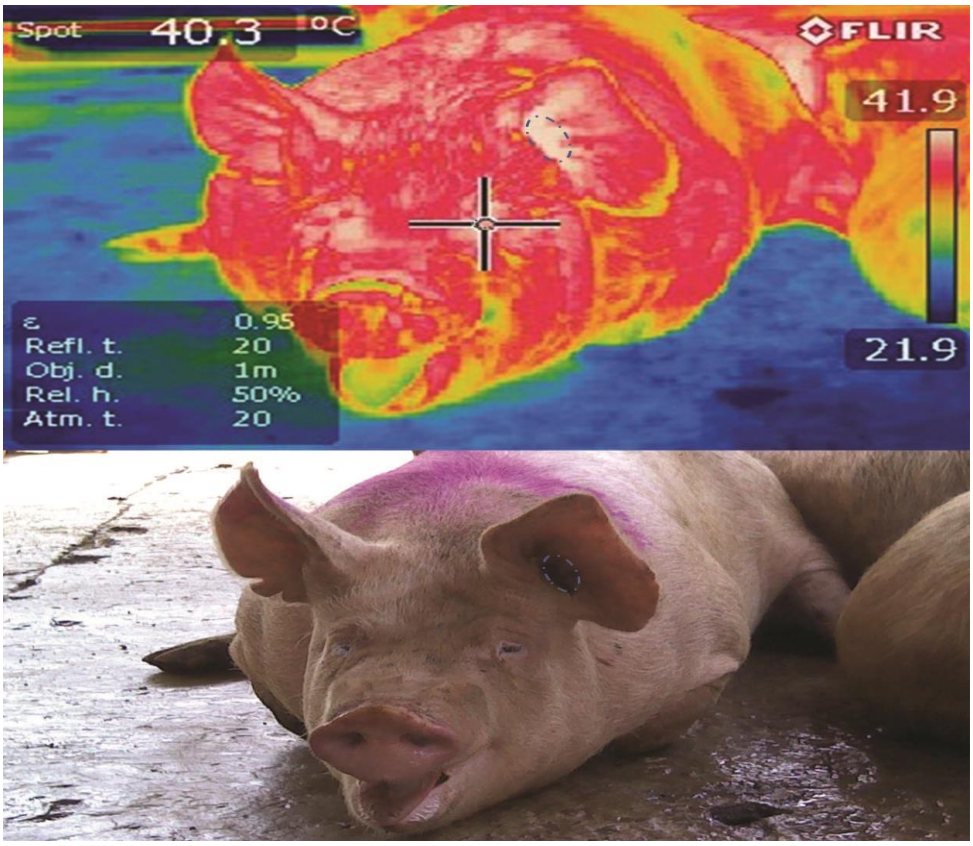

Figure 1. A pig with hyperthermia upon arrival at an abattoir (Salvador et al. 2020)

1.2 Physiological responses of market pigs to heat stress

Physiological measures are good indicators of the severity of thermal stress in animals. They can help to interpret the degree of discomfort including the nature of stressors such as hydration status, fear, muscle fatigue, nutritional/energy status. Pigs are handled throughout the process of loading, transport, unloading, lairage and stunning, which has shown to cause significant amount of stress in pigs (Mota-Rojas et al. 2006). Pigs are subjected to multiple stressors during these operations, some of which may be psychological (social mixes, overcrowding, fights, contact with humans, new surroundings) or physical (hunger, thirst, exhaustion, lesions, extreme temperature changes) (Mota-Roja et al. 2020). These stressors can significantly impact animal welfare through increased incidence of skin lesions (Becerril-Herrera et al. 2009) and deterioration of meat quality based on their duration and intensity (Becerril-Herrera et al. 2010). As the nervous system releases adrenocorticotropic hormone (ACTH), catecholamines that prepare the organism for fight or flight are released followed by the release of adrenal glucocorticoids which initiates metabolic activities (Martinez-Miro et al. 2016). Commercial pigs are adversely affected by all factors that increase their metabolic rate (Mitchell and Heffron 1982). As the severity of hyperthermia increases, hypoxia develops in peripheral tissues (pCO_2 increases, pO_2 decreases leading to dyspnea and panting) (Williams et al. 1975). As pCO_2 increases, blood glucose increases, and lactate accumulates in the body (Bonelli and Schifferli 2001). It has been shown that these events may produce hypoxia in peripheral tissues in pigs that are particularly susceptible to this condition, as well as sudden death due to acute metabolic acidosis under severe condition (Williams et al. 1975).

Ivers et al. (2002) observed that thermally stressed pigs had higher blood lactate, ammonia, sodium, potassium, cortisol, epinephrine, norepinephrine concentrations, lower liver glycogen

concentrations, lower glycolytic potential values in the *longissimus* muscle and *semitendinosus* muscle post- transport. However, Anderson et al. (2002) reported that blood pH, lactate, bicarbonate, and base-excess values of fatigued pigs returned to baseline values 2 h post-handling. Correa et al. (2014) reported an increase in blood creatine kinase and lactate concentrations in pigs transported for slaughter during winter and summer months. Elevation in glucose, lactate and hematocrit concentrations were observed in pigs transported for long duration (Mota-Rojas et al. 2006; Becerril-Herrera et al. 2010), while increases in blood cortisol and lactate levels were observed following a short duration travel (Pérez et al. 2002).

1.2.1 Cortisol

Cortisol is produced in a circadian pattern and is elevated during stressful conditions (Beuving and Vonder 1978). Mitchell and Kettlewell (1994) reported elevated blood cortisol including elevation in the heterophil to lymphocyte (H/L) ratios following transport, indicating that the HPA axis was activated during the event. Cortisol, glucose, lactate and creatine have been reported as reliable biomarkers which illustrates impact of stressors on animals transported for various purposes (Gross and Siegel 1993). Bradshaw et al. (1996b) reported that plasma cortisol profile of pigs increased after loading and remained high up to 5 h while transported, suggesting that transport could elevate the plasma cortisol levels in pigs.

Under stressful condition, HPA axis is activated to secrete corticotropin releasing hormone (CRH) from paraventricular nucleus (PVN) of the hypothalamus (Smith and Vale 2006). Consequently, CRH initiates the release of adrenocorticotropic hormone (ACTH) from anterior pituitary which acts on the adrenal cortex, causing it to release glucocorticoids (cortisol in most mammals or corticosterone in birds and rodents) (O'Connor et al. 2000). Extended high cortisol levels can

impact adversely on productive performance such as growth rate, feed efficiency, and could increase fat content in pigs (Mormède et al. 2011). Foury et al. (2007) reported a significant positive correlation between basal cortisol levels and carcass fat content. Pearce and Paterson (1993) reported increased cortisol concentration in pigs under space restrictions. Furthermore, individual coping styles (active and passive) in pigs are associated with differences in behavioural, physiological, and endocrine responses to stress (Hessing et al. 1994a). Hessing et al. (1994a) reported higher average heart rate and increased cortisol concentrations in active than passive pigs.

1.2.2 Glucose

Glucose is essential for metabolic activities in the body (Zhang et al. 2009). The muscle glucose level postmortem shows the degree of stress an animal experienced prior to slaughter. In addition, the activation of HPA axis during stressful event stimulates hepatic gluconeogenesis, proteolysis, and facilitates the breakdown of starch to release glucose (Sherwood et al. 2013).Through hepatic gluconeogenesis, amino acids and other non-carbohydrate sources are converted to glucose for metabolism in the liver (Sherwood et al. 2013). Following feed withdrawal before transport, glucogen is degraded to release glucose into the blood (Zhang et al. 2009; Sherwood et al. 2013). Gluconeogenesis ensures that blood glucose level is maintained by breaking glycogen to release glucose during fasting (Sherwood et al. 2013). Blood glucose is the major metabolic fuel and the minimum threshold must be maintained for proper functioning of the brain (Sherwood et al. 2013). The use of blood glucose is highly regulated for prioritizing to brain tissues (Sherwood et al. 2013). Through lipolysis, lipids are broken down and free fatty acids are released into the bloodstream to maintain blood glucose levels for optimum brain function (Sherwood et al. 2013). In addition,

corticosterone stimulates proteolysis especially in the muscles, which causes the break down of proteins into amino acids for gluconeogenesis or other related metabolic activities (Sherwood et al. 2013).

During transport or other related stressors, thermal load can influence the blood glucose level leading to depletion in extreme cases (Dadgar et al. 2012b). Depending on the intensity, stress alters the concentrations of blood glucose and glycogen levels in animals (Vosmerova et al. 2010; Voslářová et al. 2011). González et al. (2007) reported that blood glucose decreased following long durations of transport stress in dairy cattle. Leheska et al. (2003) observed a depletion of muscle glycolytic potential in pigs exposed to 8 h of transport while Warriss et al. (1983) did not observe a significant change in the muscle glycogen of pigs following 6 h of transport. Low glucose concentrations in muscles postmortem could lead to the development of poor meat quality.

1.2.3 Lactate

Lactate is the end-product of postmortem glycolysis (Apple and Yancey 2013). Postmortem activities in tissues includes the aerobic metabolism which turns to anaerobic glycolysis, resulting in the breakdown of glycogen stores to lactic acid leading to pH decline (Apple and Yancey 2013). Muscle pH postmortem is an important trait which determines the quality of meat (Boudjellal et al. 2008). Within 6-10 h postmortem, muscle pH declines slowly from 7.4 to 5.6-5.7 with ultimate pH of 5.3-5.7 at 24 h (Scheffler and Gerrard 2007). In addition, ultimate pH (pH24 h) postmortem is the best indicator of several important attributes of pork traits, including water-holding capacity, tenderness and color (Boler et al. 2010). Jaturasitha (2008) demonstrated that the value for early postmortem muscle pH (45 min) of normal meat is higher than 6.0, and drops to pH 5.3-6.0 by 24

h postmortem (Warriss 2000). Pale meat occurs when the pH at 45 min and 24 h postmortem is less than 6.0 and 5.3, respectively (Warriss 2000), while meat with an ultimate pH beyond 6.0, is graded as dark, firm and dry (DFD) (Adzitey and Nurul 2011).

1.2.4 Behavioral responses of pigs to heat stress

Pigs adopt some behavioral changes such as wallowing, shade-seeking, off-feed, increased water intake and increase in respiratory rate to cool the body under hot atmospheric air temperatures. During heat exposure, the HPA axis is activated and heat is generated due to an increase in catecholamines and cortisol concentrations (Schaefer et al. 2002). There is evidence of increased intake of water, reduced feed intake and limited contact with pen mates in the heat-stressed pigs (Huynh et al. 2005a). Low feed intake, increase in respiratory rate, and increase in core body and skin-surface temperatures are important indicators of heat stressed in pigs (Renaudeau et al. 2011).

There is a rapid increase in respiratory rate and rectal temperature in heat stressed pigs within the first 24-48 h, with a gradual decrease at a constant rate (Renaudeau et al. 2010). Sapkota et al. (2016) investigated the response of finisher pigs to acute heat stress. Results showed that body-core temperature of pigs increased from 38.3°C to 40.3°C after 30 minutes of exposure. In addition, average skin-surface temperature increased from 33.5 °C to 40.5 °C, with a decline in the body-core temperature when pigs were returned to thermoneutral conditions. De Oliveira et al. (2018) evaluated the effect of acute (48 h) and chronic stress (71 d) stress on finishing pigs, and reported an increase of 0.6°C in rectal temperature and 0.2°C of core body temperature, and higher respiratory rate (125.2 breaths/minute vs 86.4 breaths/minute) in short-term heat stressed pigs compared to pigs exposed to a longer period of thermal stress, respectively.

Pigs lack functional sweat glands, consequently, heat is mainly expelled from the body through a rapid increase in respiratory rate which increases heat loss through evaporation (Baumgard and Rhoads 2013). Huynh et al. (2005a) observed a rapid increase in the respiratory rate of finisher pigs exposed to air temperatures above 22.4 °C. This result is in agreement with other studies which reported optimum temperature (thermal comfort zone) of lactating sows between 16°C and 22°C (De Bragança et al. 1998; Quiniou and Noblet 1999). Huynh et al. (2005a) reported an increase in rectal temperature and respiration rate, as well as the water-to-feed ratio, with decreased feed intake when air temperature was raised from 16 °C to 32 °C. Decreasing feed intake is one of the major mechanisms of ameliorating heat load in heat-stressed animals (Quiniou and Noblet 1999; Renaudeau et al. 2011; Baumgard and Rhoads 2013).

Le Dividich et al. (1998) showed that ambient temperatures around 30°C may cause a 50% decrease in feed intake of growing and finishing pigs. Similarly, a 30% drop in feed intake was observed in young pigs kept for ~2 weeks in climatic chambers at 33°C (Collin et al. 2001). Similarly, Pearce et al. (2015) reported that keeping growing pigs at air temperatures of 35 °C for 7 d led to a 47% decrease in feed intake.

1.2.5 Molecular and cellular response to heat stress in pigs

The productive performance of pigs is grossly affected by a reduction in food intake (Black et al. 1993; Collin et al. 2001). The effect of heat stress on cell structure and function has been elucidated by several molecular biology techniques and genomics (Collier et al. 2018; Cross et al. 2018). It has been established that heat stress distorts gene expression and could cause oxidative damage (Zhang et al. 2002; Ross et al. 2017). Ma et al. (2019), in a study using transcriptome analysis of

the *longissimus dorsi*, demonstrated that heat stress downregulates the genes responsible for tissue accretion and metabolism and upregulates genes involved in DNA or protein damage/recombination. In addition, heat stress induces hepatic protein expression related with heat-shock protein response, oxidative stress response and immune defence (Cui et al. 2016).

Genome-wide association studies (GWAS) have shown that some genomic regions are connected to feeding behaviours in heat-stressed pigs (Cross et al. 2018). Kim et al. (2018) applied GWAS to evaluate the genomic basis of the physiological indicators of heat stress such as respiratory rate, rectal temperature, and skin temperature. The molecular/cellular response to heat-load exposure is generally divided into three different phases of primary importance; heat-shock protein expression (HSPs), interferon-inducible genes, and the activation of small non-specific stress responses of specific cell lines (Moran et al. 2006). Heat-shock proteins are seen as one of the most important heat-stress markers (Mayer 2005) which help in the maintenance of cellular homeostasis. Hao et al. (2016) and Seibert et al. (2018) reported an increased expression of HSPs in the gut, liver, muscle or ovary of heat-stressed pigs. Heat-shock proteins inhibit apoptosis, aid protein folding, tracking and protein complex assembly or disassembly (Hassan et al. 2019) and contribute to the modulation of the immune system and metabolism (Jing et al. 2018; Zininga et al. 2018). Heat-shock proteins act as first line of cellular defence during stressful conditions thereby safeguarding cell integrity, maintain functional signaling pathways essential for cell survival and function.

1.2.6 Effects of transportation on pig health

Transport is the last phase in the production life cycle of food animal species (Grigor et al. 2004). Ritter et al. (2009) and Sutherland et al. (2009) reported that among pigs transported for slaughter

in the USA, between 0.19 and 0.25% died in-transit, while 0.27 to 0.44% became non-ambulatory at the point of slaughter, leading to a loss of about $46 million annually in the US swine industry (Ellis et al. 2003). The number of pigs that die or become non-ambulatory at the plant post-transport has raised serious animal welfare concerns leading to enacting legislation (Council Regulation 1/2005/EC) on animal transport in the European Union (Grandin 2000). Transportation distance has been noted to impact adversely on welfare and meat quality of animals (Perez et al. 2002; Mota-Rojas et al. 2006). Additionally, cold and heat stress during transport may affect quality of meat at slaughter (O'Neill et al. 2003; dalla-Costa et al. 2006). Abbott et al. (1995) and Vecerek et al. (2006) noted that high ambient temperatures increase the morbidity and mortality of pigs during transport. Moreover, dalla Costa et al. (2006) observed significant skin bruises and discoloration in animals transported for slaughter in the winter period. Dead and non-ambulatory pigs can be caused by the combination of several biotic and abiotic factors; genetics, facility design, people, management, transportation, and processing plant (Ellis and Ritter 2005a). These losses call for; (1) improvement in the welfare of finished pigs during transport in order to prevent the occurrence of dead and non-ambulatory pigs (National Pork Board, 2007), and; (2) increased enforcement of rules and regulations (e.g. Downed Animal and Food Safety Protection Act: Bill H. R. 661 by the US House of Representatives 2007 and Bill S. 394 of the US Senate 2007) to reduce dead or non ambulatory pigs. Non-ambulatory pigs are pigs which show different degrees of physical challenges (eg. cripples, slows etc) at the packing plant post-transport (Anderson et al. 2002). Fatigued and injured pigs are typical examples of non-ambulatory pigs (Ellis and Ritter 2005a). Fatigued pigs are those pigs that are free from serious injuries, trauma, or disease, but are unable to move with other pigs after transport (Ritter et al. 2005). Furthermore, injured pigs are those pigs that have serious structural deformations due to stress and other limitations from a

transport system (eg. nature of truck, compartment or animal interaction) which reduce mobility at the slaughter house (Ellis and Ritter 2005b).

Transport has a tremendous effect on muscular postmortem changes and overall meat quality. Carr et al. (2005) investigated fresh pork characteristics of 246 fatigued pigs post-transport, and reported that stressed pigs had poor pork (high pHu of 6.0, low Minolta L* values of 45.72, and low drip losses of 1.91%). Consequently, Carr et al. (2005) suggested that meat quality of fatigued pigs depend on transport distance in the production cycle. Gregory (1994) reported that short-term stress prior to slaughter increases the rate of postmortem metabolism, leading to the development of PSE pork. In addition, Anderson et al. (2002) and Ritter et al. (2005) noted that fatigued pigs at the slaughter house exhibited open-mouth breathing (44%), skin discoloration (77%), muscle tremors (83%), and abnormal vocalizations (30%).These studies validated transport as a potential stress factor which is capable of altering the biochemical composition of muscles with negative effects on meat characteristics.

1.3 Meat quality classification

Stress during handling and transport and nutritional status have a direct impact on pork quality. The meat pH, color, water holding capacity and other meat biochemical attributes postmortem depend on the pre-slaughter condition of animals. Meat can be classified based on these attributes which can be altered depending on the intensity of stress or other environmental conditions prior to slaughter. In reference to ultimate pH (pHu), color (L* value) and water-holding capacity (WHC) or drip loss, meat can be traditionally classified into reddish-pink, firm and non-exudative (RFN), PSE or DFD, as illustrated in Table 1.1. Other meat quality categories such as reddish-pink, soft and exudative (RSE) and pale, firm and non-exudative (PFN) meats (Kauffman et al.

1993; Van-Laack and Kauffman 1999) have also been identified. Reddish-pink, firm and non-exudative pork represents the optimal quality classification and is characterized by a pHu of 5.6-5.8, reddish-pink color, Minolta L* value of 42-50 and drip loss of 2-5% (Warner et al. 1997; Joo et al. 1999; Correa et al. 2007). When pigs are exposed to extreme stressful conditions or other chronic stress related muscular disorders, RFN pork could change to PSE or DFD (Warriss 2010).

There are other meat quality classifications apart from the definitions of Correa et al. (2007). For example, Chae et al. (2007) and Sellier and Monin (1994) reported that PSE pork has a pH value less than 6.0 at 1 h postmortem and 5.5-5.7 at 24 h postmortem. These differences could have resulted from the use of different equipment and protocols, different meat samples, time, age of animal, season and the prevailing atmospheric conditions. Other studies showed that PSE pork has a pH value less than 6.0 at 1 h postmortem and 5.5-5.7 at 24 h postmortem respectively (Sellier and Monin 1994; Chae et al. 2007), drip loss greater than 5 % and a Minolta (L*) value greater than 50 (Warner et al. 1997; Joo et al. 1999). Dark, firm and dry pork is known for its high pHu (> 6.0), a low drip loss (< 2%) and Minolta color (L*) value lower than 42 (Correa et al. 2007). These meats have short storage life due to their susceptibility to postmortem microbial growth (Gill 1976). Pale, firm and non-exudative pork is firm (normal structure) as in RFN pork with a pHu of 5.5–5.8 (Correa et al. 2007) and Minolta color (L*) greater than 50, as in PSE meat (Kauffman et al. 1992; Correa et al. 2007). The RSE pork has normal pHu 5.6-5.8 (Correa et al. 2007), reddish-pink color L* value 42-50; (Warner et al. 1997; Correa et al. 2007) with a high drip loss as in PSE pork (>5 %) (Warner et al. 1997; Joo et al. 1999). Pale firm and normal and RSE pork are the most commonly observed meat quality defects in Canada (Murray 2001; Faucitano et al. 2010) which account for about 13-47% of meat quality defects compared to PSE (13-21 %) and

DFD pork (2-10%).

1.3.1 Meat pH

The pH change is noteable postmortem activities in muscle tissues. Meat pH postmortem depends on the concentrations of lactate, muscular physiologic condition at stunning and the muscular energy production rate (Immonen et al. 2000). Generally, muscle pH is determined one hour (pHi) and/or 24 h (pH24) after slaughter (Barton-Gade et al. 1996). The overall values for pH 1h and pH 24h in pork loin muscle after slaughter are 6.3-6.7 (National Pork Producers Council 2001) and 5.5-6.0 (Warriss 2010) respectively.

Table 1.1. Pork quality classification* according to pHu, drip loss (DL) and objective color (L*).

Quality class	pHu	DL	L*
PSE	<5.5	>5%	>50
PFN	5.5 – 5.8	<5%	>50
RSE	5.6 – 5.8	>5%	42-50
RFN	5.6 – 5.8	<5%	42-50
DFD	> 6.1	<2%	≤42

*PSE =Pale soft and exudative, PFN=Pale firm and normal, RSE =Red soft and exudative, RFN =Red firm and normal, DFD =Dark, firm and dry. Modified from Correa et al. (2007)

1.3.2. Meat color

The color and appearance of fresh meat are major deciding factors in purchasing meat by consumers (Brewer et al. 2002). Consumers associate bright red color with freshness in raw meat, and grey or tan color with cooked meat (Cornforth and Jayasingh 2004). Myoglobin is the primary meat pigment, although low levels of blood haemoglobin may also be found (Cornforth and Jayasingh 2004). There are variations in the muscle myoglobin content among species, breed, sex, age, muscle fibers, and depending on the biochemical state of iron present (Fe^{2+} or Fe3+) (Ledward 1992). According to Stewart (1965), myoglobin is present in the muscle under three main forms:

deoxymyoglobin (Mb^{2+}), oxymyoglobin (OMb^{2+}) and metmyoglobin (MMb^{3+}), each one providing a different color to meat. The interconversion of myoglobin forms depends on pigment (myoglobin and hemoglobin) concentration, chemical states, stress exposure, and rate of oxygenation and oxidation (Klont et al. 1998; Lawrie 2002). The chemical state of myoglobin affects the meat color and other biochemical properties.

There are both subjective and instrumental approaches used for the measurement of pork color. Subjective pork color evaluation can be determined either using the Japanese Pork Color Standards (JCS; ranging from 1: extremely light to 6: extremely dark) (Nakai et al. 1975), the Canadian Pork Quality Standard, which includes six colour levels (Maignel et al, 2012), or the National Pork Producers Council Pork Quality Standards (NPPC 1999). The NPPC color evaluation standards range from 1 (pale pinkish gray to white) to 6 (dark purplish red). The instrumental or objective color assessment is based on the colorimetric scale CIE L*, a*, b* (International Commission on Illumination 1986). The L* value shows the degree of lightness, 100 for white (a complete reflection), and 0 for black (complete absorption of light). The a* value shows the intensity of redness or greenness, since red and green colors are complementary, with a range of -60 (pure green) to +60 (pure red). Lastly, the b* value indicates yellowness (or blueness), and ranges from -60 (pure blue) to +60 (pure yellow).

1.3.3 Drip loss

Drip loss refers to inability of fresh meat to retain its natural juices in the muscle and muscle fibers (Rasmussen and Andersson 1996). A 2-5% drip loss is considered normal, while values below or above could be sign of meat deterioration (Murray 2001). Drip loss or purge is a red liquid consisting of about 85% water and 10% protein (Savage et al. 1990). Other chemical

components of muscle such as glycolytic enzymes, amino acids and water-soluble vitamins can be found in the purge (Savage et al. 1990). The degree of drip loss in meat is governed by both intrinsic and extrinsic factors. The intrinsic factors include genotype, breed, sex, age, muscle type and location within muscle and postmortem rate and extent of pH decline (Huff-Lonergan and Lonergan 2005). The postmortem muscle acidification is directly related to meat color (Brewer et al. 2001) and WHC (Joo et al. 1999; Schäefer et al. 2002) due to its effects on protein structure and hydration properties. Enfält et al.(1997) and Offer (1991) reported that a decrease in pH close to the isoelectric point causes the shrinkage of most myofibrillar proteins leading to meat with low pHu, reduced WHC and lighter color (Hammelman et al. 2003). The shrinkage of myofibrillar proteins due to myosin denaturation increases the rate of purge or drip loss leading to meat paleness (Monin 2004). Other metabolic factors affecting the WHC of meat are the postmortem muscle temperature, the degree of muscle shortening (Honikel et al. 1986), and the breakdown of intermediate filaments and cytoskeletal proteins (Huff-Lonergan and Lonergan 2005) during *rigor mortis* development.

Excessive denaturation of myofibrillar proteins affect the protein's ability to bind water and results in a poor WHC (Offer and Knight 1988). Increases in purge loss in meat have been linked with the postmortem degradation of proteins such as talin, vinculin (Bee et al. 2004) and desmin (Davis et al. 2004; Huff-Lonergan and Lonergan 2005). In addition, the degradation of membrane proteins one hour postmortem leads to the formation of drip walls and a reduction in the water holding capacity of muscle tissues (Lawson et al. 2004). Genetic effects such as mutations in the ryanodine receptor due to the halothane gene (ie Porcine Stress Syndrome [PSS]) is one of the important intrinsic factors associated with excessive purge/drip loss in pork (Fujii et al. 1991). Based on the metabolic characteristics of fiber types, fast white fibers (eg. loin or *longissimus* muscle) tend to

be more involved in the anaerobic postmortem conditions, resulting in higher protein denaturation with a negative impact on meat color and drip loss (Ryu and Kim 2005; Choe et al. 2008). However, muscles with higher amounts of slow red fibers (eg. ham or *semitendinosus* muscle) and low proportions of fast white fibers are linked with low levels of lactate accumulation one hour postmortem, a higher protein solubility and corresponding darker muscle with lower drip losses (Choe et al. 2008). Extrinsic factors such as nutrition, animal rearing conditions, pre-slaughter handling and carcass handling (chilling, cutting, boning, slicing and retail packing) have been identified to increase drip loss in pig muscles (Huff-Lonergan and Lonergan 2005). Pigs raised under free-range conditions produced meat with less drip loss than pigs raised under intensive systems (Lambooij et al. 2004; Pugliese et al. 2005). In addition, there was an increase in the water-holding capacity of meat from pigs raised in an enriched environment, compared with pigs under conventional system (Klont et al. 2001). The methods of pre-slaughter animal handling (Schäefer et al. 2002; Hambrecht et al. 2005) and postmortem carcass handling (Hambrecht et al. 2004a) have also been reported to exert different effects on meat quality. Vander-Wal et al. (1999) reported a reduced water holding capacity 24 h postmortem in pigs subjected to a short-term acute stress prior to stunning.

1.3.4 Meat tenderness

Tenderness is an attribute, which customers consider while categorizing meat. Among the major sensory traits (appearance, juiciness, flavor, taste, and tenderness), tenderness is rated as a decisive factor which affects the quality of meat. Meat chewing and eating quality is a combination of appearance, flavor, tenderness, and juiciness (Aaslyng et al. 2003; Pereira and Vicente 2013). Poor animal welfare during transportation and rough pre-slaughter handling may affect adversely on

carcass and meat quality, leading to a significant economic loss (Grandin 2007). In addition, tenderization mainly applies to red meat (beef, mutton, horse, pork, buffalo and lamb) as a result of high toughness and least applied to white meats like chicken and fish (Bekhit et al. 2014).

The Warner–Bratzler shear force (WBS) is a scientific tool for quantifying meat tenderness, measured in Newtons (N). Meat tenderization involves the degradation of collagen in both quantity and type (Veiseth et al. 2004), a reduction in the diameter of muscle fibre bundles (Renand et al. 2001) and changes in the sarcomere length during *rigor mortis* (Rhee et al. 2004) which occur due to chemical and structural changes during aging. The development of meat tenderness depends on the structure, connectivity of the skeletal muscle tissue and the proteolytic enzyme activities (MacBride and Parrish 1977). Wang et al. (2013) reported an increased endogenous enzymatic activity due to actomyosin dissociation stimulated by heat supply when meats are cooked. Light et al. (1985) reported the conversion of collagen to gelatin at 80 °C and shrinkage of collagen at 60–70 °C. However, Barbanti and Pasquini (2005) observed that high temperatures could result in increased moisture loss and hardening of myofibrillar proteins leading to tougher meat.

1.4 Conclusion

There has been a technological advancement in developing remote sensors for measuring body temperatures of animals in the last decade. Generally, most of these emerging methods for body temperature measurement are automated or semi-automated. These new technologies have the potentials of great economic benefits to pig producers and packers. Digital infrared imaging is one of the non-invasive technological tools currently under study for monitoring animal welfare. Infrared technology enables simple and real-time remote temperature measurement in animals and

could contribute significantly to improving animal welfare and meat quality. Adoption of digital infrared temperature capturing in a commercial setting could aid a remote temperature data acquisition, thus minimizing the exposure of animals to handling stress, which is common with the use of the invasive temperature measuring tools such as the mercury bulb thermometer, implantable and other wireless sensor network devices. The application of digital infrared imaging on-farm and at the packing plant could help to improve animal welfare and meat quality.

1.5 Objectives

The overall objective of this study was to evaluate infrared technology as a tool for identifying market pigs at risk of transport stress, death loss, and traits related to PSE meat. Specific objectives were to:

1. Evaluate the reliability of consumer and research grade infrared digital cameras for measuring temperature change in market pigs.
2. Determine the effects of a controlled handling treatment on ocular and body temperature in market pigs.
3. Evaluate the reliability of consumer grade digital infrared camera for detecting temperature change in market pigs pre- and post-transport.
4. Determine best region (ocular or body) for digital infrared imaging in market pigs pre- and post-transport.
5. Determine the relationship between pig temperature perimortem and pork quality in market pigs.
6. Determine the relationship between pig temperature and physiological markers of stress.

1.6 Hypotheses

The overall hypothesis for this study is that digital infrared technology is capable of identifying stressed or febrile pigs pre- and post-transport. Specific hypotheses include:

1. A digital infrared consumer grade camera (≤$1,000) and a digital infrared research grade camera (≈$15,600) would give similar ocular and body temperature measures in pigs.
2. Ocular and body regions would measure different temperatures in response to a mild handling stress in pigs.
3. Transportation induces a physiologic change in market pigs.
4. Compared to pigs with low temperatures, pigs with high temperatures on-farm would show higher temperatures post-transport.
5. Pigs with higher temperatures post-transport would show a prevalence of PSE meat traits.
6. Pigs with higher temperatures post-transport would show high levels of blood cortisol with low levels of blood glucose and lactate at sticking.

2.0: Comparison of two digital infrared thermographic cameras for recording temperature in market pigs

This chapter examined two digital infrared thermographic cameras for recording ocular and body temperatures in market pigs**.** The study also evaluated the effects of a controlled handling treatment on ocular and body temperature in market pigs. Digital infrared thermographic (DT) cameras come with different pixel resolutions: the research grade (RG) DT camera has high pixel resolution of 320×240 and is more costly (~$16,000 CDN), while the consumer grade (CG) DT camera has lower pixel resolution of 80×60 and is more affordable (~$1,000 CDN)**.** The study investigated if both DT cameras could accurately measure ocular and body temperatures for health assessment in pigs. The less expensive CG camera could be adopted by packers if it has similar temperature measures compared to the expensive RG camera for welfare assessment in pigs.

2.1 Abstract

The objective of this study was to evaluate the reliability of two digital infrared [research grade (RG) and consumer grade (CG): FLIR Systems, Inc.] cameras for measuring ocular and body temperatures in market pigs. A total of 168 finisher pigs (111.5±13.2 kg BW) were used in this study. The control group (84 pigs) received no handling treatment except for minimal handling within the pen during infrared (IRT) imaging. The handling treatment group (84 pigs) were exposed to a controlled handling treatment which involved moving 2 or 3 pigs down the hallway and back to the home pen (a distance of approximately 100 m). Three ocular and body images were collected from the body/flank and eye region of the pigs; prior to the handling treatment, and again after handling, using the two digital infrared cameras. FLIR® software was used to download the thermographic images from both cameras. Average baseline and post-handling temperatures were obtained from each pig. Data were analyzed using Pearson's correlations and linear regression models in SAS (SAS 9.4) to determine the relationships between temperatures collected by both cameras. Mixed models (SAS 9.4) were used to determine effect of control and handling treatments and sampling time points on ocular and body temperatures. Ocular and body temperatures increased post-handling, and there were significant differences between baseline temperatures and post-handling temperatures. Only the ocular measures showed treatment × time interaction showing the effects of handling treatment. Body temperatures showed better correlations between RG and CG cameras than ocular. The cameras gave similar results and were positively correlated ($r=0.93$, $P<0.001$), indicating that the cheaper camera could be suitable for measuring pig temperatures.

Keywords: Infrared camera, ocular temperature, body temperature, handling stress, pigs

2.2 Introduction

About 0.69% of market pigs transported for slaughter either become non-ambulatory or are dead on arrival (DOA) at the packing plant annually (Ritter et al. 2009; Agriculture and Agri-Food Canada 2018). This represents a significant economic loss, as well as an important welfare concern in the pork industry. Transport is a known stressor, and all pigs should be fit prior to transport in order to endure normal transport conditions. Diseased or compromised pigs are most likely to become fatigued, injured, non-ambulatory or die during and/or after transport. Furthermore, pig handling and post-transport stress could have deteriorating effects on pork quality (Schwartzkopf-Genswein et al. 2012).

The Code of Practice for Care and Handling of Pigs identifies fever as one of the main indicators of a compromised pig, and declared febrile pigs as being unfit for transport (National Farm Animal Care Council 2014). However, febrile or diseased pigs are not easily identified on-farm prior to transport due to the use of unreliable/difficult temperature testing techniques for disease diagnoses. Invasive diagnostic tools like rectal thermometers and thermal microchips have been in use for decades by farmers and researchers for detecting temperature in pigs. However, these methods are characterized by their inaccurate measures (Godyn and Herbu 2017), time-consuming (Johnson et al. 2011), and for inducing stress in pigs (Loughmiller et al. 2001). This led to a concern to identify safe and non-invasive alternatives for recording pig temperature in the pork industry.

Infrared thermography is a remote sensing method for body temperature measurement. Infrared thermography measures alterations in heat production and loss due to changes in blood flow arising from environmental stress or diseases (McManus et al. 2016). Infrared thermography has been used to explore thermoregulatory processes in human medicine (Jones 1998) and for detecting health

and welfare issues in animals (Polat et al. 2010; Schaefer et al. 2012).

Monitoring pig temperature using digital IRT could help in identifying febrile pigs (Cook et al. 2015). If producers could identify sick or stressed animals easily using IRT, they could respond quickly to retain and treat sick animals, or modify handling procedures and other transport conditions to reduce stress and mortality during and after transport. However, previous studies using IRT have failed to agree on the best region (eye, body, neck or ear region) for accurate IRT measurement in animals (Banhazi et al. 2009; Weschenfelder et al. 2012). In addition, the high cost of this technology poses a barrier to its adoption by the pork industry. Digital infrared cameras are produced with different pixel resolutions; an expensive RG digital infrared camera with high pixel resolution costs approximately (~$16,000) CDN, whereas the less expensive CG digital infrared camera with lower pixel resolution costs approximately $1000 CDN. If the less expensive CG digital infrared camera can detect temperatures in market pigs similar to the more expensive RG infrared digital camera, it could be a useful tool for producers and packers to aid in the detection and early treatment of sick/febrile pigs, and to reduce morbidity and mortality.

The aim of this study was to compare the performance of two digital infrared cameras: a research grade camera with high pixel resolution (~$16,000 CDN), and a less expensive consumer grade camera (~$1000 CDN) with lower pixel resolution, for recording temperature in market pigs.

The specific objectives of this study were to;

1. Compare the ability of the consumer grade and research grade infrared digital cameras for measuring ocular and body temperature in market pigs.
2. Determine the effects of a controlled handling treatment on ocular and body temperatures in market pigs.

2.3 Materials and methods

2.3.1 Experimental design

The experimental protocol for this experiment was approved by the University of Saskatchewan Animal Research Ethics Board (UCAC**:** 20190060) and all pigs were cared for according to the Code of Practice for the Care and Handling of Pigs (NFACC 2014). The experiment was performed at the Prairie Swine Centre, Saskatoon, SK from June to August 2019.

2.3.2 Animals and housing

One hundred and sixty-eight (168) finisher pigs at near market weight (111.5±13.2 kg, Yorkshire × Landrace, PIC Camborough Cross genetics) were enrolled in this study. Pigs were housed in two semi-intensive rooms at the Prairie Swine Centre in groups of 4 or 5 pigs per pen (2.36m × 1.68 m, fully slatted). Pigs were weighed during the trial week and individually marked prior to testing using a spray marker for identification.

2.3.3 Treatment and infrared measures

Eighteen pens of 4-5 pigs were randomly assigned to one of two treatments. Half of the pigs (84) acted as the Control group, which received no handling treatment other than minimal handling within the home pen to collect IRT images. The remaining 84 pigs were enrolled in the Handling treatment and received a mild handling stressor consisting of moving groups of 2 or 3 pigs down the hallway and back to the home pen (a distance of approx. 100 m). Digital thermographic images (Figures 2.1 and 2.2) were obtained on the whole body/flank and eye area of all pigs at two time points. Baseline ocular and body temperatures were taken on both the control and handling

treatment pigs at the home pen prior to mild handling stress and another ocular and body temperatures were taken on both groups after exposing the treatment pigs to a mild handling stress using both Research grade (RG: FLIR A325SC, Flir Systems USA, Boston, MA) and Consumer grade (CG: FLIR C3) digital infrared cameras. At each time point, three images were taken in rapid succession from each of the regions (ocular and body) using both cameras. The pigs remained in the pen during image collection and images were obtained at a relatively constant distance from the ocular and body (flank/skin side) regions. One handler entered the pen and moved each pig into a fixed position using a handling board, another technician controlled the two cameras from the alleyway outside the pen, and a third technician collected data from the RG camera on a computer situated in the alleyway. Body images were obtained at a distance of approximately 2 m from the pig, and eye images were obtained from less than 1 m using both cameras (Figures 2.1 and 2.2). The emissivity setting was at 0.98 for all measurements on both cameras, as this level has been validated for accurate infrared skin measurements on swine (Soerensen et al. 2014). After the baseline measurement, handling treatment pigs were moved down a ~50 m hallway (total distance of 100 meters, 5-10 minutes) in groups of two or three (two groups per pen). Pigs were then returned to their home pen and ocular, and body surface temperature measurements were repeated using both cameras within 5 minutes of completing the handling stressor. Control pigs remained in their home pen throughout the trial. Approximately one hour after baseline measurements were recorded, temperature measurements were repeated on Control pigs.

2.3.4 Temperature data extraction

FLIR® software (FLIR Systems OU, Estonia) was used to download the thermographic images from the digital infrared cameras. Temperatures were read from the region of interest (eyes or

body/flank) by drawing a box around it. The temperature and standard deviation within the box were read directly from the FLIR® software, and recorded as either the body or ocular temperatures depending on the region observed. The ocular temperatures were read by drawing a box around the eye and its immediate surroundings (not the whole face). The average temperature within the box was recorded as ocular temperature for each image. This process was repeated for the three ocular thermographic images for each pig and the mean and SD were calculated. If one of the readings varied by >1 SD it was excluded from analysis. Body temperatures were read by drawing a rectangular box on the body image (covering at least 75% of the skin/flank area) of each pig. The average temperature and SD within this region was recorded as body temperature for each image. The mean body temperature was calculated from the three body images. If one of the three readings varied by >1 SD it was excluded from analysis.

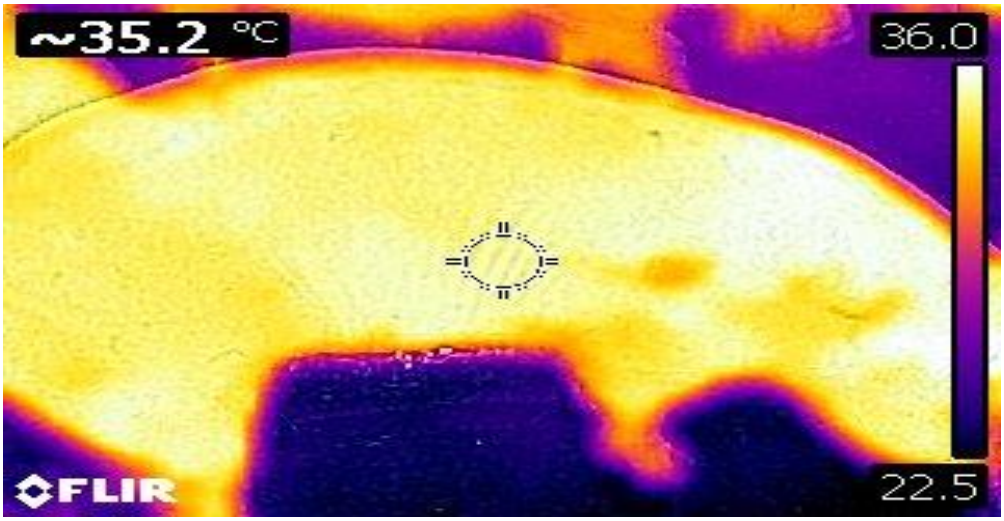

Figure 2.1 Body infrared digital thermographic image of a pig.

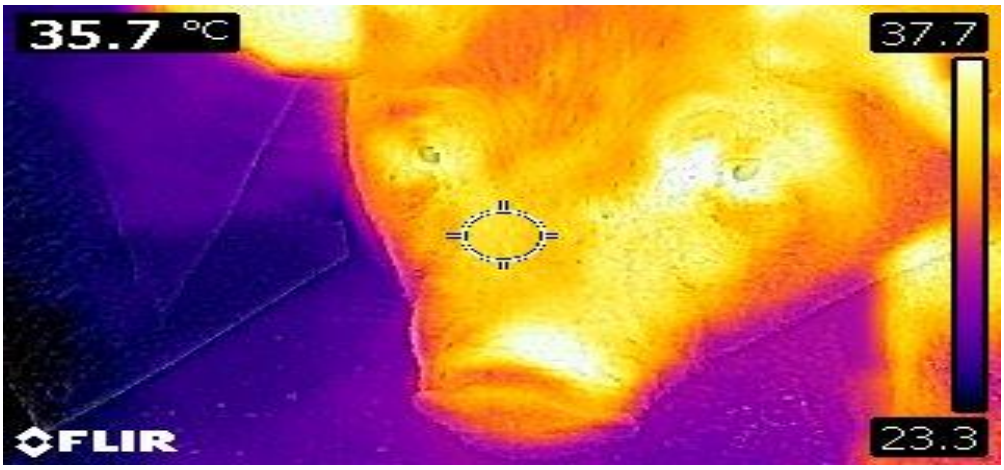

Figure 2.2 Ocular infrared digital thermographic image of a pig.

2.3.5 Statistical analysis

Pearson correlations in SAS (SAS 9.4, SAS Institute Inc., Cary, NC) were used to study the relationships between ocular and body temperatures in both cameras. Mixed models in SAS (SAS 9.4) were used to evaluate the effects of treatment (Control and Handling) and sampling time on ocular and body temperatures for each camera. Main effects in the model were treatment, time and treatment by time interaction while pen was the random effect. If $P \leq 0.05$, differences were considered significant, and if $P \leq 0.10$, trends were noted.

2.4 Results

Descriptive statistics for baseline infrared temperatures (ocular and body regions) of Control and Handling treatment groups recorded with each camera are shown in Table 2.1, while Table 2.2 shows the descriptive statistics for infrared temperatures measured post-handling.

2.4.1 Comparison of consumer grade and research grade cameras

The Pearson correlation coefficients (r, P-values and [n]) comparing baseline infrared ocular and body temperatures of the two infrared cameras are shown in Table 2.3. There were positive correlations (r=0.67, P<0.001) between baseline CG and RG ocular temperatures of the Control group. Positive correlations (r=0.50, P<0.001) were also found between pre-handling CG and RG ocular temperatures of the treatment group. Also, strong positive correlations (r=0.93, P<0.001) were found between baseline CG and RG body temperatures in the Control group (Table 2.3), and a similar observation (r=0.83, P<0.001) was found between pre-handled CG and RG body temperatures of the treatment group.

The Pearson correlation coefficients (r, P-values and [n]) for post-handled (Handling treatment pigs) infrared ocular and body temperatures of the two infrared cameras are shown in Table 2.4. There were positive correlations (r=0.60, P=0.001) between CG and RG ocular temperatures in the post-handled control group, and similar relationships (r=0.50, P=0.001) were found between CG and RG ocular temperatures in the Handled group. A strong positive correlation (r=0.83, P=0.001) was found between body temperatures recorded using CG and RG cameras in the Control group. Similarly, there was significant agreement between body temperatures across CG and RG cameras (r=0.70, P=0.001) in the Handled group.

2.4.2 Effects of controlled handling treatment on ocular and body temperatures

LS Means for treatment and time effects in the mixed model analysis of body and ocular temperatures for the CG and RG infrared digital cameras are shown in Table 2.5. There were significant main effects of treatment and time for ocular and body temperatures, and significant treatment by time interactions for ocular temperatures in both cameras. Handled pigs had higher

temperatures than Control pigs, and temperatures measured at time two were consistently higher. With both cameras, the SEM value for ocular temperature was approximately half as large as that for body temperature.

There were treatment by time interactions for the CG and RG ocular temperatures (Fig 3 and 4 respectively). For the CG camera (Fig 3), ocular temperature for Control and Handled pigs was not significantly different at time 1 but differed at time 2. The ocular temperatures of handled pigs were higher than those of control pigs ($P<0.05$). Similarly, ocular temperature for Control and Handled pigs were not significantly different at time 1 but differed at time 2 for the RG camera (Fig. 4). The ocular temperatures of handled pigs were higher than the control pigs ($P<0.05$).

Table 2.1: Descriptive statistics for baseline infrared ocular and body temperatures in Control and Handling treatment pigs.

Variable	N	Mean Temperature (°C)	Std Dev	Min.	Max.
Consumer Grade Camera					
Ocular:					
Control group	84	36.33	0.91	33.70	37.90
Handling Treatment group	84	36.60	0.72	34.80	38.30
Body:					
Control group	84	33.39	1.73	28.80	36.80
Handling Treatment group	84	34.20	1.22	31.30	37.10
Research Grade Camera					
Ocular:					
Control group	84	36.32	1.02	33.40	37.90
Handling Treatment group	84	36.62	0.70	34.70	38.00
Body:					
Control group	84	32.83	2.11	27.00	36.50
Handling Treatment group	84	33.50	1.44	29.80	36.70

N=Number of animals, Std Dev = Standard deviation, Min.=Minimum temperature, Max.= Maximum temperature.

Table 2.2. Descriptive statistics for post-handling infrared ocular and body temperatures in Control and Handling treatment pigs.

Variable	N	Mean Temperature (°C)	Std Dev	Min.	Max.
Consumer Grade Camera					
Ocular:					
Control group	84	36.80	0.80	34.40	38.20
Handling Treatment group	84	37.80	0.63	36.00	38.90
Body:					
Control group	84	34.34	1.50	31.10	37.80
Handling Treatment group	84	35.25	1.14	31.90	37.40
Research Grade Camera					
Ocular:					
Control group	84	36.51	0.90	34.10	37.90
Handling Treatment group	84	37.40	0.69	35.80	38.90
Body:					
Control group	84	33.56	1.80	29.10	37.00
Handling Treatment group	84	34.30	1.28	30.80	36.70

N=Number of animals, Std Dev = Standard deviation, Min.=Minimum temperature, Max.= Maximum temperature.

Table 2.3. Pearson correlation coefficients (r, P-values and [n=168]) for baseline infrared ocular and body temperature measures recorded on Control and Handling treatment* pigs using consumer grade (CG) and research grade (RG) infrared cameras.

	CG Camera				RG Camera			
	Control		Handling Treatment		Control		Handling Treatment	
	Ocular	Body	Ocular	Body	Ocular	Body	Ocular	Body
CG								
Ocular Control	1.00	0.62 <0.001	0.30 0.007	0.30 0.009	0.67 <0.001	0.50 <0.001	0.50 <0.001	0.35 0.001
Body Control		1.00	0.21 0.052	0.38 0.001	0.70 <0.001	0.93 <0.001	0.60 <0.001	0.60 <0.001
Ocular Handling Treatment			1.00	0.50 <0.001	0.08 0.500	0.10 0.370	0.60 <0.001	0.24 0.03
Body Handling Treatment				1.00	0.31 0.004	0.40 0.001	0.51 <0.001	0.83 <0.001
RG								
Ocular Control					1.00	0.80 <0.001	0.52 <0.001	0.58 <0.001
Body Control						1.00	0.61 <0.001	0.67 <0.001
Ocular Handling Treatment							1.00	0.62 <0.001
Body Handling Treatment								1.00

*Control: pigs remained in their home pen for the entire study (Baseline and Post- treatment evaluations). Handling treatment: pigs were evaluated in the home pen (Baseline), then underwent a mild handling stressor before Post- treatment measures were collected.

Table 2.4. Pearson correlation coefficients (r, P-values and [n=168]) for post-handling infrared ocular and body temperature measures recorded on Control and Handling treatment* pigs using consumer grade (CG) and research grade (RG) infrared cameras.

	CG Camera				RG Camera			
	Control		Handling Treatment		Control		Handling Treatment	
	Ocular	Body	Ocular	Body	Ocular	Body	Ocular	Body
CG								
Ocular Control	1.00	0.53 <0.001	0.30 0.013	0.37 0.001	0.60 0.001	0.50 0.001	0.40 0.001	0.30 0.003
Body Control		1.00	0.24 0.03	0.31 0.004	0.60 0.001	0.83 0.001	0.50 0.001	0.33 0.002
Ocular Handling Treatment			1.00	0.40 0.002	0.20 0.11	0.20 0.10	0.32 0.003	0.30 0.02
Body Handling Treatment				1.00	0.50 0.001	0.50 0.001	0.40 0.001	0.70 0.001
RG								
Ocular Control					1.00	0.74 <0.001	0.50 <0.001	0.45 <0.001
Body Control						1.00	0.49 <0.001	0.44 <0.001
Ocular Handling Treatment							1.00	0.63 <0.001
Body Handling Treatment								1.00

*Control: pigs remained in their home pen for the entire study (Baseline and Post treatment evaluations). Handling treatment: pigs were evaluated in the home pen (Baseline), then underwent a mild handling stressor before Post treatment measures were collected.

Table 2.5 LS Means for treatments and time points from analysis of body and ocular temperatures recorded using consumer and research grade cameras.

Item	Treatment*		Time**		SEM	P- value		
	Control	Handled	1	2		Trt.	Time	Trt.xTime
Consumer grade camera:								
Ocular temp. (°C)	36.55	37.20	36.47	37.29	0.064	<0.001	<0.001	<0.001
Body temp. (°C)	33.86	34.71	33.78	34.79	0.126	<0.001	<0.001	0.498
Research grade camera:								
Ocular temp. (°C)	36.41	36.99	36.47	36.94	0.074	<0.001	<0.001	<0.001
Body temp. (°C)	33.19	33.88	33.16	33.91	0.153	0.006	<0.001	0.879

*Control: pigs remained in their home pen for the entire study (Baseline and Post treatment evaluations). Handling treatment: pigs were evaluated in the home pen (Baseline), then underwent a mild handling stressor before Post treatment measures were collected.

**Time 1= baseline temperatures, Time 2= post-handling temperatures

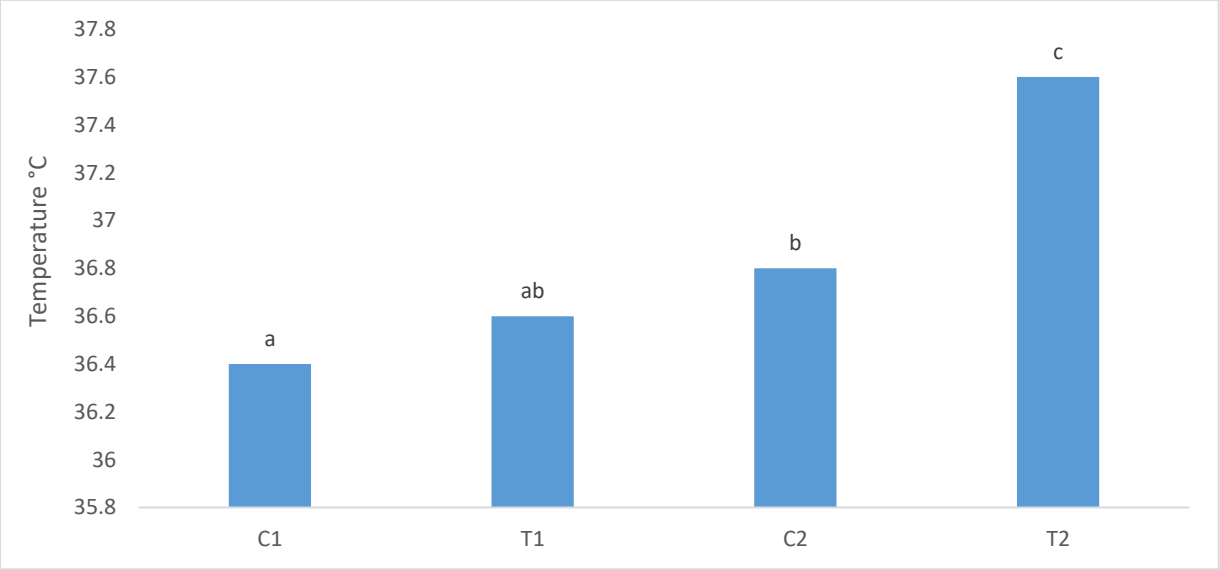

Figure 3. Treatment by time interaction for ocular temperatures recorded using the consumer grade camera. C1= Control treatment at time 1; T1= Handled treatment at time 1; C2= Control treatment at time 2; T2= Handled treatment at time 2.

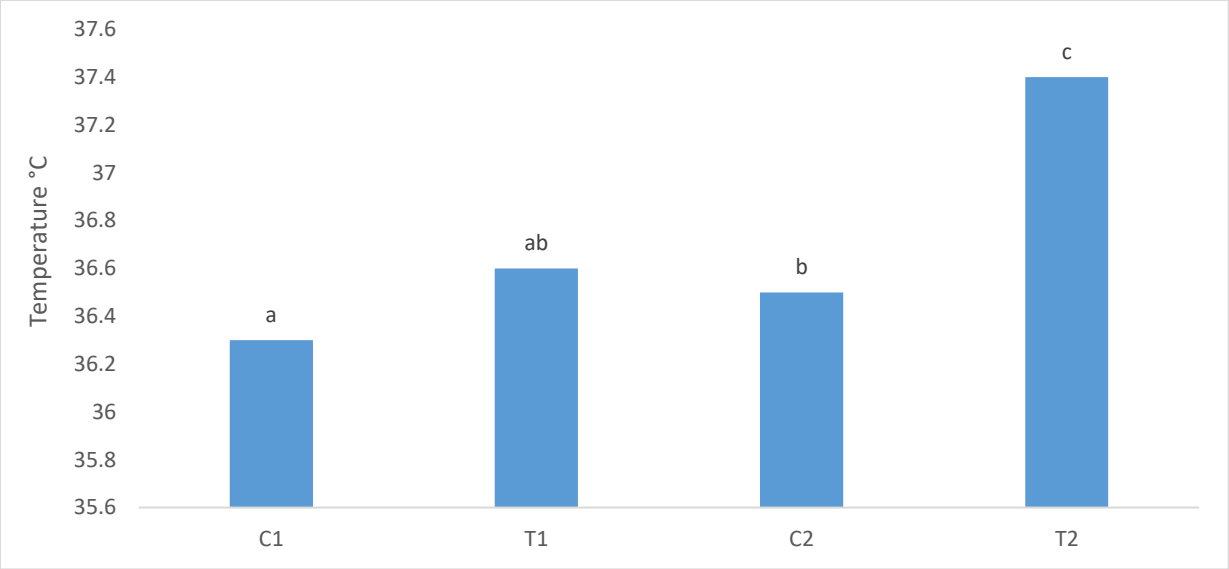

Figure 4. Treatment by time interaction for ocular temperatures recorded using the Research grade camera. C1= Control treatment at time 1; T1= Handled treatment at time 1; C2= Control treatment at time 2; T2= Handled treatment at time 2.

2.5 Discussion

2.5.1 Comparison of CG and RG digital infrared cameras

Safe methods for measuring temperatures in pigs on-farm are needed for improved animal health and welfare. Infrared thermography is a non-invasive imaging technique for recording the superficial thermal radiations from a body (Turner 2001). The superiority of IRT (non invasive, non stressful, and quick measurement display) compared to mercury bulb thermometric devices for body temperature measurements was established by Loughmiller et al. (2001) thus IRT was advocated for safe and accurate surface body temperature measurements in animals.

Baseline and post-handled IR temperatures were correlated in both cameras. Correspondingly, strong positive correlations were found between CG and RG body temperatures in the control group, and likewise agreement between CG and RG body temperatures in the Handled group. This indicates that the less expensive consumer grade camera (~$1,000) CDN and the expensive research grade camera (~$16,000) CDN can produce similar ocular and body temperature measures in pigs. The adoption of the CG camera by producers and packers for disease or stress surveillance in pigs could potentially improve animal welfare and lower cost of production.

2.5.2 Effects of controlled handling treatment on ocular and body temperatures

Mild handling on-farm could subject pigs to stressful conditions capable of increasing their body temperatures, thus, raising a serious welfare concern. In the current study, significant treatment effects and interactions with time were found in both cameras. Handled pigs had higher temperatures than controls for all measures. Temperatures measured at time 2 (post-handling temperature) were consistently higher than the time 1 (baseline temperatures) measures.

In the CG camera, ocular temperature for control and handled pigs was not significantly different at time 1 but it was at time 2 measures. Similar observation was found in the RG camera were ocular temperature for control and handled pigs were not significantly different at time 1 but significant at time 2 measures. This implies that handling treatment exposed the pigs to a mild stress which might have led to an increase in ocular and body temperatures of the pigs. Schaefer et al. (2002) reported that stress stimulates the hypothalamic–pituitary–adrenocortical axis to generate heat, thus causing elevation in the core body temperature of animals. Additionally, Knízková et al. (2007) reported that IRT could detect a slight change in body temperature of animals following stressful events.

The ocular temperatures were higher than body temperatures at both time points and with both cameras. Soerenson and Pederson (2015) revealed that the subcutaneous fat deposits in mature pigs could influence body surface temperatures, which could explain why body temperatures were lower than ocular temperatures in this study.

Body temperatures showed higher positive correlations across cameras than ocular temperatures, suggesting that the body could be a more precise location for recording temperatures in pigs. This study showed that IRT is capable of measuring ocular and body temperatures in pigs. In addition, handling and human-animal interactions on-farm were found to increase temperature of pigs which could discomfort pigs thus, impairing animal welfare.

2.6 Conclusions

This study hypothesized that the less expensive consumer grade camera would give similar temperature measures compared to the more expensive RG camera. The data showed that the

results from the consumer and research grade digital infrared cameras were highly correlated, with body temperatures giving better r values than ocular measures.

The less expensive CG camera is more affordable for measuring pig temperatures/detecting health problems and could be more beneficial to the pork industry if it can be adopted for use by producers/packers. In addition, pig temperatures increased at time 2, when pigs were handled, but only ocular measures showed the treatment by time interactions possibly because timing of T2 measure was too soon or maybe handling stress was too mild. Even though eye temperatures were higher than the body temperatures, body temperatures showed better correlation between cameras than ocular temperatures, although both correlations were significant. Only the ocular region showed a clear treatment by time interaction, showing the effects of handling treatment.

At this initial stage, IRT shows potential for measuring temperatures in pigs. Overall, the CG camera could be suitable for temperature monitoring of pigs in the swine industry. Pigs responded to handling with a measurable increase in ocular temperatures, and there were different strengths for the ocular and body regions in capturing infrared measures.

3.0: Evaluation of market pigs using a digital infrared camera pre- and post-transport

The previous study compared the research grade (RG) and the consumer grade (CG) digital infrared cameras for measuring mild handling stress in market pigs. Temperature measures from the two cameras were significantly correlated, thus, making the adoption of the less expensive CG camera economically feasible for monitoring temperature change and health assessment in market pigs. In this experiment the CG camera was used to compare pig temperatures pre- and post-transport in market pigs.

Transport is a known stressor, and pigs transported for slaughter are often received with injuries or different pathological conditions. Difficulty in breathing or open-mouth breathing, blotchy skin (irregular skin blanching and erythema), refusing to move on approach, trembling, and cases of dead on arrival (DOA), dead in pen (DIP), non-ambulatory injured (NAI), and non-ambulatory non-injured (NANI) pigs have been reported at the abattoir following the transport of market pigs for slaughter. These abnormalities result in significant economic losses to the pork industry and could be abated if market pigs were screened prior to transport to ensure that only healthy pigs are selected for transport. The goal of this chapter was to determine if thermographic images obtained on-farm were predictive of pigs' response to transport. The study monitored pre- and post-transport temperatures to examine the effects of transport on pig welfare prior to slaughter. In addition, thermograms of the ocular and body regions were compared to determine the best region for infrared thermographic imaging in market pigs pre- and post-transport.

3.1. Abstract

This study investigated if pig temperatures recorded on-farm were predictive of temperatures post-transport. A total of 120 market pigs (BW=105.1 ± 4.9 kg) were selected and transported in five replicates (20-25 pigs/replicate) for ~2.5 hr to an abattoir during summer of 2020. Infrared thermographic (IRT) images of two body areas (ocular and body regions: three images per pig) were obtained on all pigs at three time points (T1: after selection, weighing and tagging on-farm three days pre-transport, again at T2: one day on-farm pre-transport, and at T3 in lairage post-transport). Infrared thermographic images were collected with minimal handling using the CG digital infrared camera. Data collected were analyzed using Pearson correlations and linear regression models in SAS (SAS 9.4) to determine if thermographic images obtained on-farm were predictive of pigs' response to transport. No relationship (P>0.05) was found between on-farm temperatures (T1 and T2) and post-transport temperatures (T3), an indication that on-farm temperatures were not predictive of post-transport temperatures. Open mouth breathing, blotchy skin, and shoulder lacerations were observed in some pigs post-transport. Positive correlations (r=0.28, P<0.01) were found between T1 and T2 body temperatures, while T1 and T2 ocular temperatures showed a tendency for correlation (r=0.18, P=0.09), indicating that body region could be better for on-farm infrared thermometry in pigs. In addition, regression analyses showed strong positive associations (r^2= 0.80, P=0.01) between T1 and T2 body temperatures. This study found that on-farm IR temperature measures were not predictive of IR temperatures measured post-transport in market pigs. Possible reasons for this negative finding are discussed.

Keywords: Pigs, transport, welfare, abattoir, stress response, infrared, body temperature

3.2 Introduction

Stress resulting from handling of pigs on-farm, during transport and before slaughter can impact the behaviour and welfare of pigs (Kim et al. 2004) increase morbidity and mortality (Rademacher and Davies 2005), and affect pork quality (Dalla-Costa 2006). According to transport surveys, 0.19-0.25% of market pigs transported for slaughter die during transport, while about 0.27-0.44% become non-ambulatory post-transport, costing the US swine industry approximately $46 million per annum (Ritter et al. 2009; Sutherland et al. 2009). Similarly, a $4 million loss was reported in Canada due to death and injuries (13,000 deads or 0.08%) during transport of pigs to slaughter (Schwartzkopf-Genswein et al. 2012). These losses could be reduced with the introduction of a tool for on-farm screening and identification of stressed, febrile or diseased pigs prior to transport. Disease transmission on-farm and post-transport could be reduced if this tool could identify diseased pigs for isolation and treatment prior to transport.

Infrared thermography (IRT) is a non-invasive technique which allows the recording of body temperature of animals (Stewart et al. 2005; Warriss et al. 2006). Infrared thermography could be helpful in assessing early health challenges and stress responses in pigs on-farm and prior to slaughter (Alsaaod et al. 2014). It may be particularly useful for identifying compromised market pigs prior to transport, or at the packing plant before processing. Moreover, based on the close associations between muscle temperature change and early postmortem pH decline (Klont and Lambooij 1995), IRT could be useful for predicting and addressing meat quality traits such as pale soft and exudative (PSE) pork.

A study in human medicine revealed that IRT showed high levels of repeatability (Petrova et al. 2018) and reproducibility at pre-determined anatomical regions of interest (Ammer 2008).

However, there is controversy over the precise body region (ocular or body/skin region) that is the most accurate for recording temperature changes in market pigs. Schaefer et al. (2004) reported that IRT images of the ocular region showed earliest response to disease in pigs. Weschenfelder et al. (2013) found that the IRT ocular temperature of pigs prior to slaughter was a promising measurement for predicting variations in important meat quality traits. Banhazi et al. (2009) reported an inconsistent IRT temperature reading obtained from the skin/body of growing pigs and concluded that the skin/body region might not be ideal for IRT imaging in pigs due to the presence of dirt, hair, or water on the skin, which can influence the level of radiation emitted from the body surface.

This study compared the digital thermographic images of market pigs before and after transport to determine if IRT images collected on-farm were predictive of IRT temperatures post-transport. The study also evaluated two anatomical regions; the body (skin/flank) and ocular region, to determine which region is better for infrared thermographic imaging in market pigs.

The objectives of this study were to determine;

1. Effects of transport on animal welfare.
2. If on-farm temperatures were predictive of post-transport temperatures in pigs.
3. If ocular or body region is better for digital infrared imaging in market pigs.

3.3 Materials and methods

3.3.1 Experimental design

The experiment was conducted during the 2020 summer (July-August 2020) at the Prairie Swine Centre (PSC), Saskatoon, SK. One hundred and twenty market pigs (live weight 105.1 ± 4.9 Kg, Yorkshire × Landrace, PIC Camborough Cross genetics) were used for this study. The experimental protocols for this experiment were approved by the University of Saskatchewan Animal Research Ethics Board (UCAC: 20190060). The pigs were born and raised to market weight at the PSC. They were housed in finisher pens in groups of 5 pigs per pen (2.36 m × 1.68 m, fully slatted) and on reaching market weight (100 kg) were transported to a federally inspected packing plant in Saskatchewan for slaughter.

3.3.2 Animal selection and identification

Five groups of market pigs were selected based on live weight and fitness from the finishing herd. Only healthy market pigs were selected for transport. Each group included 20 – 25 animals, with a total of 120 pigs shipped (77 gilts and 43 barrows). Pigs were selected three days before transport, weighed, ear tagged, and tattoed with an individual number for identification at the abattoir.

3.3.3 Infrared data collection on-farm

Digital thermographic infrared images of two body areas (ocular and body regions: three images for each per pig) were obtained on all selected pigs in their home pen immediately after selection, weighing and tagging (T1: three days prior to transport) using a CG infrared camera (FLIR C3). Ocular and body temperatures were recorded again on the day before transport (T2), this time with

minimal handling. The purpose of collecting IRT images on-farm at two time points was to determine which temperatures (following handling, or of calm pigs) would be more predictive/show a stronger relationship with temperatures after transport. Infrared images of the whole body (lateral image) were obtained from a 2 m distance from each pig. The ocular IRT images were obtained from a 0.25 m distance from each pig following the protocol adapted from Weschenfelder et al. (2013) and Stewart et al. (2008). The emissivity was set at 0.98 for all images according to Weschenfelder et al. (2013).

3.3.4 Transportation of pigs

Selected pigs were transported in weekly batches (20-25 pigs/week) from the PSC to a commercial packing plant in southern Saskatchewan, two and half hours drive away (distance ~225 km). Pigs were transported on the main deck of a commercial livestock trailer bedded with wood shavings at a space allowance of approximately 0.7 m^2/pig.

3.3.5 Unloading and infrared data collection at the plant

On arrival at the plant, the fitness of animals was assessed visually during unloading and in lairage pens. Pigs were unloaded and moved into a single lairage pen. Handlers at the abattoir used shaker paddles to move the pigs, and pigs walked along a wide alley on solid concrete floors to the lairage pen. The time taken to unload the trailer (start and end) as well as any handling problems were recorded. Infrared measurements were repeated on all pigs immediately after they entered the lairage pen. Infrared measures were obtained from the pig's whole body/flank and eye region using the CG digital camera. As in the on-farm temperature measurements, three images from each of the body and ocular regions of individual pigs were taken in rapid succession using the CG camera.

The pigs remained in the lairage pen during image collection and images were obtained at different distances from the pigs, depending on the region of interest. Digital infrared images were obtained from a distance of approximately 2 m from the pigs' body, and 0.25 m away from the eye region.

3.3.6 Temperature data extraction

The infrared thermographic images were downloaded using Flir software (FLIR Systems OU, Estonia). Temperatures were read from the region of interest (eyes or body/flank) by drawing a box around the digital thermographic ocular or body images. The temperature and standard deviation of the selected area were read directly from the Flir software. The ocular temperatures were read by drawing a box around the eye and its immediate area (not the whole face). The average temperature within the box was recorded as ocular temperature for each image. This process was repeated for the three ocular thermographic images for each pig and the results from the three replicates were averaged. Body temperatures were read by drawing a box on the body image (skin/flank) of each pig. The average temperature within this region was recorded as body temperature and the three body temperatures were then averaged to get the final body temperature for each pig. If one of the three readings of the ocular or body region varied by >1 SD it was excluded from analysis.

3.3.7 Statistical analyses

Pearson correlations and linear regression models in SAS (SAS 9.4) were used to test for associations between pre- and post-transport temperatures. Regression models included one temperature time point (T1 or T2) as the predictor with later temperature measures (T2 or T3) as the outcome. Agreement between ocular and body temperatures across observation times (T1, T2,

and T3 measures) was used to determine if one region (ocular or body) was more suitable for detecting temperature change in market pigs pre- and post-transport. If P≤0.05, differences were considered significant, and if P ≤0.10, trends were noted.

3.4 Results

3.4.1 Evaluation of market pigs prior to transport using IRT

The minimum and maximum temperatures during loading and transport days in Saskatoon, and pig conditions at loading are shown in Table 3.1. Pigs all appeared healthy prior to transport. The minimum and maximum temperatures during transport days at the packing plant, and pig condition observed at unloading are shown in Table 3.2. Open mouth breathing and blotchy skin were observed in some pigs especially in replicates 2, 3, and 5, which had maximum temperatures of 26°C, 27°C and 34°C respectively. The descriptive statistics for infrared ocular temperature of the market pigs on-farm and post-transport are shown in Table 3.3. The T1 average ocular temperature (OT1: ocular temperature on-farm 3 days before transport) was numerically higher compared to ocular temperatures at T2 (one day before transport) and T3 (after transport) time points. Pearson correlation coefficients (r, P-values and [n]) of pre-and post-transport infrared ocular temperature are shown in Table 3.4. There were no significant correlations (P>0.05) among ocular temperatures, but there was a tendency for a positive correlation (r =0.18, P =0.09) between ocular temperatures at times 1 and 2. The descriptive statistics for infrared body temperature pre-and post-transport are shown in Table 3.5. The T1 average body temperature (BT1: body temperature on-farm 3 days before transport) was numerically higher than body temperatures at T2 (one day before transport) and T3 (after transport) time points. Table 3.6 shows the Pearson correlations (r,

P-values and [n]) of pre-and post-transport infrared body temperatures. There was a significant correlation (r =0.28, P<0.01) between body temperatures at times 1 (BT1: body temperatures taken on-farm 3 days before transport), and 2 (BT2= body temperatures taken on-farm a day prior to transport). The Pearson correlation coefficients (r, P-values and [n]) between body and ocular temperatures at same time points are shown in Table 3.7. There were positive correlations (r=0.65, P <0.01) between ocular and body temperatures at same time points. The linear regression of infrared ocular and body temperatures (Table 3.8) within time points showed significant positive associations at each time (T1: r^2=0.33; T2: r^2=0.31; T3: r^2=0.43, P <0.01). The linear regression analysis also showed significant positive associations between body temperatures at T1 and T2 (r^2=0.80, P <0.01), but not between ocular T1 and T2 temperatures (r^2=0.03, P=0.09).

Table 3.1. Minimum and maximum ambient temperatures during the transport days in Saskatoon*, and pig condition observed at loading (7:00-10:00 AM).

Date	Replicate	Min. temp. (°C)	Max.temp. (°C)	Pig condition/ behavior at loading
July 20, 2020	1	14.90	16.40	Good
July 27, 2020	2	11.40	21.90	Good
August 3, 2020	3	17.90	24.20	Good
August 10, 2020	4	10.80	19.50	Good
August 17, 2020	5	12.60	24.40	Good

*Source: https://climate.weather.gc.ca. Min.temp=Minimum temperature, Max.temp.=Maximum temperature.

Table 3.2. Minimum and maximum ambient temperatures during the transport days at the packing plant*, and pig condition observed at unloading (10:00 AM-3:00 PM).

Date	Replicate	Min. temp. (°C)	Max.temp. (°C)	Pig condition/ behavior at unloading
July 20, 2020	1	17.10	17.70	Good
July 27, 2020	2	21.20	25.20	Heavy breathing, blotchy skin
August 3, 2020	3	22.70	27.40	Open mouth breathing, hematoma
August 10, 2020	4	23.10	26.10	Good
August 17, 2020	5	24.90	33.00	Open mouth breathing, blotchy skin

*Source: https://climate.weather.gc.ca. Min.temp=Minimum temperature, Max.temp.=Maximum temperature.

Table 3.3: Descriptive statistics of infrared ocular temperatures of the market pigs on-farm and post-transport

Variable*	N	Mean Temperature ± SD (°C)	Min.	Max.
OT1**	95	36.78 ± 0.83	34.10	38.37
OT2	120	35.36 ± 0.99	32.43	37.13
OT3	120	36.51 ± 0.94	33.93	38.77

** OT1=ocular temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. OT2=ocular temperatures taken on-farm a day prior to transport and OT3 =ocular temperatures taken in lairage pens at the packing plant post-transport.Min.=Minimum température, Max.=Maximum temperature.

Table 3.4. Pearson correlations (r, P-values and [n]) of pre- and post-transport infrared ocular temperatures of market pigs.

Variable*	OT1	OT2	OT3
OT1	1.00 (95)	0.18 0.09 (95)	-0.03 0.78 (95)
OT2		1.00 (120)	-0.07 0.43 (120)
OT3			1.00 (120)

* OT1=ocular temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. OT2=ocular temperatures taken on-farm a day prior to transport and OT3 =ocular temperatures taken in lairage pens at the packing plant post- transport. Min.=Minimum temperature, Max.=Maximum temperature.

Table 3.5: Descriptive statistics of infrared body temperatures of the market pigs pre- and post-transport.

Variable*	N	Mean Temperature ± SD (°C)	Min.	Max.
BT1**	95	36.19 ± 0.96	33.87	38.33
BT2	120	34.69 ± 1.21	30.30	36.83
BT3	120	35.90 ± 1.32	32.00	38.70

** BT1=body temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. BT2=body temperatures taken on-farm a day prior to transport and BT3 =body temperatures taken in lairage pens at the packing plant post- transport. Min.=Minimum temperature, Max.=Maximum temperature.

Table 3.6. Pearson correlations (r, P-values and [n]) of pre- and post-transport infrared body temperatures of market pigs. Significant correlations are indicated in bold.

Variable*	BT1	BT2	BT3
BT1**	1.00 (95)	**0.28** 0.01 (95)	0.15 0.14 (95)
BT2		1.00 (120)	0.05 0.59 (120)
BT3			1.00 (120)

** BT1=body temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. BT2=body temperatures taken on-farm a day prior to transport and BT3 =body temperatures taken in lairage pens at the packing plant post- transport. Min.=Minimum temperature, Max.=Maximum temperature.

Table 3.7. Pearson correlations (r, P-values and [n]) of pre- and post-transport infrared body and ocular temperatures recorded at same time points. Significant correlations are indicated in bold.

Variable*	OT1	OT2	OT3
BT1	**0.58** <0.01 (95)	0.14 0.18 (95)	0.10 0.45 (95)
BT2		**0.55** <0.01 (120)	-0.03 1.00 (120)
BT3			**0.65** <0.01 (120)

.* BT1=body temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. BT2=body temperatures taken on-farm a day prior to transport and BT3 =body temperatures taken in lairage pens at the packing plant post- transport. OT1=ocular temperatures taken on-farm three days before transport, n = 95 as infrared measures were not collected in Replicate 5. OT2=ocular temperatures taken on-farm a day prior to transport and OT3 =ocular temperatures taken in lairage pens at the packing plant post-transport.

Table 3.8. Linear regression of infrared ocular and body temperatures of market pigs on-farm and at the packing plant. Significant results are indicated in bold.

Variable*	Estimate	N	SE	R sq	T value	Pr > |t|
OT1 vs OT2	0.15	95	0.09	0.03	1.73	0.090
OT1 vs OT3	-0.03	95	0.09	0.01	-0.27	0.780
OT2 vs OT3	-0.07	120	0.09	0.01	-0.79	0.430
BT1 vs BT2	0.22	95	0.78	0.80	2.86	**0.010**
BT1 vs BT3	0.12	95	0.08	0.02	1.48	0.140
BT2 vs BT3	0.05	120	0.08	0.03	0.54	0.590
OT1 vs BT1	0.50	95	0.07	0.33	6.78	**<0.010**
OT2 vs BT2	0.46	120	0.06	0.31	7.22	**<0.010**
OT3 vs BT3	0.51	120	0.05	0.43	9.50	**<0.010**

*OT1=ocular temperatures taken on-farm three days before transport, OT2=ocular temperatures taken on-farm a day prior to transport, OT3 =ocular temperatures taken in lairage pens at the packing plant post transport, BT1=body temperatures taken on-farm three days before transport, BT2= body temperatures taken on-farm a day prior to transport and BT3=taken in lairage pens at the packing plant post transport.

3.5 Discussion

3.5.1 Evaluation of market pigs prior to transport using IRT

The identification of unfit pigs prior to transport is one of the most important welfare issues related to pig transport (Grandin 2017). Pigs unfit for transport are most likely to become fatigued, injured, non-ambulatory or die during transport. The number of in-transit deaths in pigs indicates the extent to which animal welfare was compromised on-farm and during transport (Malena et al. 2007). This is because the rearing system and other associated farm conditions have a significant influence on welfare and health condition of pigs (Scott et al. 2006). Other reasons for in-transit deaths in market pigs could be on-farm health problems which were compounded by transport stress (Lebret et al. 2006; Gade 2008). Apart from physical injuries (eg. lameness, shoulder sores, necrotic prolapses, hernia, abscesses) which can be identified easily, the identification of injuries/disease conditions with internal pathology (eg. pneumonia, swine erysipelas, swine fever etc) in market pigs on-farm is very challenging. Infrared thermography has been shown to detect febrile responses to systemic illness, bovine viral diarrhoea (Schaefer et al. 2004) and respiratory disorders in cattle (Schaefer et al. 2007) and could potentially identify unfit market pigs on-farm prior to transport.

Change in temperature is one of the easiest ways of detecting deviations from the normal state of health in both humans and animals. Extreme temperatures could result in loss of a herd or lower animal productivity. There is need to monitor pig temperatures on-farm in order to ensure optimum productivity. The ocular and body temperatures at time 1 were higher than ocular and body temperatures at times 2 and 3. T1 temperatures were measured on-farm when pigs were agitated following selection, weighing, ear tagging, tattooing, and collection of infrared measures, while T2 temperatures were measured when pigs were calm a day prior to transport. The T3 temperatures

were measured in lariage post-transport. The higher ocular and body temperatures at time 1 suggests a change in the physiologic condition of the pigs following the on-farm handling which could raise a serious welfare concern. It implies that certain routine farm practices such as selection, weighing, ear tagging, tattooing, and change of pen could trigger a significant change in body temperatures of pigs. Grandin (2001) and Pajor (2000) reported that selection, weighing, tattooing and movement of finishing pigs from the farm to the packing plant for slaughter could elicit stress in pigs. Hentzen et al. (2012) reported that handling and restraining pigs on-farm could increase the body temperature of pigs which is consistent with the result of this study. Friendship et al. (2009) reported that the temperature of group of pigs in a pen recorded using a hand-held infrared camera was able to predict illness in pigs two days prior to mortality in the pen. This study was run during July and August 2020, to capture the effects of maximum temperatures on market pigs, however, the temperature range within this period were 10.80-24.00°C during transport and 17.10-33.00°C on arrival at the packing plant. Thus, depending on the day, pigs likely experienced heat stress that resulted in open mouth breathing (20%) and blotchy skin (5%) observed while in lairage. Anderson et al. (2002) reported open mouth breathing and erythema in market pigs transported for slaughter during summer months. This finding confirms that transport can increase pig temperatures thereby endangering pig welfare under severe conditions. In addition, this study noted that IRT could potentially detect febrile pigs on-farm prior to transport. Infrared thermography could be beneficial for monitoring pig temperatures in order to improve animal welfare on-farm and prior to transport.

The on-farm (T1 and T2) temperatures collected in this study were not predictive of post-transport (T3) temperatures. However, there were positive correlations between times 1 and 2 body temperatures. This indicates that IRT could detect temperature differences in market pigs on-farm,

with the body region showing a better sensitivity than the ocular region for detecting temperature differences in pigs. Contrary to Schaefer et al. (2004), who found the ocular region was most reliable for infrared thermographic imaging in pigs, this study shows that the body region may be more suitable for detecting a temperature change in pigs on-farm and prior to transport. In contrast, Banhazi et al. (2009) observed inconsistent IRT body temperatures in growing pigs and concluded that the skin/body region might not be ideal for IRT imaging in pigs. The inconsistent body temperatures could have been due to the wetness of pigs' skin prior to IRT body temperatures measures. This could account for the differences in the IRT body temperatures as reported by Banhazi et al. (2009) and the IRT body temperatures found in this current study.

The lack of relationship found between pre- and post- transport temperatures may have been due in part to limitations of this study. Homeostasis maintains pigs' body temperature within a very small range making it difficult to obtain a strong correlation under normal health conditions. The small number of pigs used in this study also did not permit the evaluation of a wide range of infrared thermographic values. Moreover, the prevailing ambient conditions at the time of this study (July–August 2020) were insufficient to impose significant heat stress on the pigs during transport. This could account for the absence of correlations between time 1 and 3 temperatures, and time 2 and 3 temperatures. Alternatively, pigs' responses on-farm may differ from those during transport, due to the different types of stress experienced. More studies with increased number of pigs under more varied environmental conditions could improve the efficacy of IRT for measuring temperature in pigs pre- and post-transport.

3.6 Conclusion

Across time, there was a positive correlation between time 1 and 2 body temperatures, and a tendency for correlation between time 1 and 2 ocular temperatures. However, there were no significant correlations between on-farm temperatures (T1 or T2) with post-transport temperatures (T3) for either the ocular or the body region. This shows that body temperature could give a better prediction of temperature change in market pigs pre-transport/on-farm. It was hypothesized that transportation would induce a physiologic change in market pigs, resulting in higher temperatures post-transport. However, the average time 1 ocular and body temperatures were higher than temperatures recorded at time 2 and 3, suggesting that transport stress was moderate compared to the handling stress at time 1. The higher temperatures found at time 1 confirms the result from Chapter 2 indicating that pig handling is capable of increasing body temperature of pigs on-farm.

4.0: Prediction of stress physiology and pork meat quality using infrared technology

The condition of animals perimortem to a large extent determines the quality of meat at slaughter. An increase in the body temperature of pigs prior to slaughter could have a deteriorating effect on pork quality. This chapter examines the relationships between IR temperatures (ocular and body) collected in lairage post-transport and physiological and carcass characteristics, especially traits associated with PSE meat, postmortem. If IRT can predict meat quality it could be used to identify stressed pigs prior to slaughter, and management interventions could be implemented to ameliorate the adverse effects of transport stress, resulting in benefits to pig welfare and meat quality.

4.1 Abstract

This study was conducted to determine the relationships between pig temperatures in lairage, stress physiology at slaughter and meat quality traits postmortem. A total of 120 market pigs (BW=105.1 ± 4.9 kg) were transported in five groups (20-25 pigs/replicate) for ~2.5 hr to an abattoir during summer 2020. Ocular and body temperatures of the pigs were recorded using a CG digital infrared camera (FLIR C3) in lairage immediately after transport. Thermographic images were taken from 0.25 m and 2 m away from pigs' eyes and body (back/flank), respectively. At exsanguination, blood samples were collected from each pig for cortisol, glucose and lactate analyses. Carcass pH measures were taken at 1 and 3 h postmortem, and loin samples were collected for meat quality (ultimate pH, color, drip loss and tenderness) assessment. Pearson correlations and linear regression models were used to test for associations between post-transport temperatures, physiological measures and carcass quality. Meat samples were classified into four categories based on color (L*), pH and drip loss. Mixed model analysis was used to determine relationships between meat quality classifications and ocular and body temperatures. Results showed positive associations between pig temperatures and cortisol levels (ocular: r =0.40, P= 0.01; body: r =0.22, P= 0.02). Negative associations were found between post-transport temperatures and glucose levels (ocular and body: r =-0.30, P =0.01). Meat yellowness (b*) was associated with increase in temperatures (r =0.34, P =0.02). Higher ocular and body temperatures were associated with tougher meat (r = 0.50, P <0.01). Pigs that produced loins with pale soft and exudative (PSE), moderately pale soft and exudative (MPSE), pale firm and normal (PFN) meat quality had significantly (P=0.013) higher body temperatures compared to those with normal meat quality, whereas ocular temperature showed no relationship with meat quality traits. Automation of

infrared technology at the packing plant would aid in identifying pigs that are liable to yield poor carcasses, allowing for management interventions prior to slaughter to improve meat quality.

Keyword: Ocular temperature, body temperature, infrared technology, stress physiology, pork quality, pigs

4.2 Introduction

Heat stress affects not only physiological changes and growth of farm animals, but also meat quality characteristics such as pH, water retention, and meat color (Ma et al. 2015) resulting in economic loss in the livestock industry (Cobanovi´c et al. 2020). This loss is worrisome given the need to increase animal products by 40% to meet up with the animal protein requirement of the projected 40% increase in the world's population by 2050 (Food and Agriculture Organization 2021).

Meat quality depends to a large extent on nutritional status, stress physiology, genetics, and muscular activity pre- and post-slaughter (Dingboom and Weijs 2004; Ryu and Kim 2005). Four important quality traits for fresh meat include color, water-holding capacity, texture and fat content (Joo et al. 2013). Postmortem metabolism following the slaughter of animals is characterized by a change from aerobic metabolism to anaerobic glycolysis, during which glycogen breaks down to form lactic acid with a consequent pH decline in muscle tissues (Apple and Yancey 2013). The rate at which this biochemical activity proceeds depends on the health status of the animal and pre-handling stress prior to slaughter.

At slaughter, there is a change in muscle pH from 7.4 to about 5.6-5.7 within the first 10 hours, resulting in an ultimate pH (pH24 h) of 5.3-5.7 (Scheffler and Gerrard 2007). The early muscle pH (1-10 h) postmortem is used to characterize fresh meat (Huff-Lonergan and Lonergan 2007). Pale, soft, and exudative meat occurs when meat pH is less than 6.0 at >1 h postmortem (Chae et al. 2007). Pale, soft, and exudative traits may occur in meats due to acute stress prior to slaughter. According to Owens et al. (2009), acute heat stress perimortem induces rapid muscle glycogenolysis, causes increased lactic acid build-up in adipose tissues with a corresponding

decrease in early muscle pH in hot carcasses. This biochemical change results in PSE meats characterized by lower water holding capacity (WHC) and poor processing yield (Adzitey and Nurul 2011; Freitas et al. 2017).

There is a relationship between WHC and pH. Water accounts for about 75% of the total weight of meat (Apple and Yancey 2013). Water-holding capacity is the ability of meat to retain its metabolic water despite the application of external force during cutting, pressing, grinding, packaging, curing, or thermal treatment (Hamm 1960). Loss of water (drip loss) from meat can occur through evaporation, storage, thawing and cooking (Apple and Yancey 2013). Over 50% prevalence of high purge or drip loss was found in pork from pigs that were acutely stressed perimortem (Huff-Lonergan and Lonergan 2005). There is an associated pH decline following a decrease in WHC in muscle tissues (Huff-Lonergan and Lonergan 2007). A decrease in WHC results in the decreased ability of myofibrilar proteins to bind with water (den-Hertog-Meischke et al. 1997) causing shrinkage and denaturation of the myofibrillar proteins (Scheffler and Gerrard 2007). Driploss represents the inability of meat to retain the natural juices in the muscle (Rasmussen and Andersson 1996). Pork of this quality (low ultimate pH and high drip loss) results in economic loss of about $5/carcass (Murray 2001).

Meat color and appearance are reliable indicators of meat quality and freshness (Brewer et al. 2002). Bright red is associated with fresh meat, grey or tan with cooked meat, while other colors could be indicators of poor yield (Cornforth and Jayasingh 2004). In addition, PSE meat is known for its paleness with high L* value (Cheah et al. 1984).

Currently, there is limited information on methods for identifying individuals or groups of pigs which are likely to yield poor carcasses postmortem. The application of IRT at the packing plant

could be used to monitor pig temperatures and may be predictive of stress and meat quality traits. This information could be used in management pre-mortem to improve pork yield and meat quality. Therefore, the objectives of this study were to;

1. Determine relationships between pig temperatures measured at the packing plant using IRT and physiological indicators of stress.
2. Determine relationships between pig temperatures measured at the packing plant using IRT and pork quality.
3. Compare IRT measures collected from ocular and body regions of the pig to determine which region provides a better prediction of stress and pork quality.

4.3 Materials and methods

4.3.1 Experimental design

The experimental protocols for this experiment were approved by the University of Saskatchewan Animal Research Ethics Board (UCAC**:** 20190060). The infrared measures, blood collection and early postmortem pH, serum glucose and lactate evaluations took place at a commercial packing plant in Saskatchewan, while serum cortisol analysis and 24 h meat quality evaluations took place at Prairie Diagnostics and the Meat Science laboratory at the University of Saskatchewan.

4.3.2 Animal selection and identification

Five groups of market pigs (N=120, live weight 105.1 ±4.9 Kg; Yorkshire × Landrace, PIC Camborough Cross genetics) were sourced from the Prairie Swine Centre in Saskatoon (PSC), SK, and shipped to a commercial abattoir for slaughter during summer months (July-August) in 2020.

Replicate groups included 20–25 animals per group, with a total of 77 female and 43 barrows selected based on body weight. Pigs were selected at the PSC three days before transport, and were weighed and given ear tags and individual slap tattoos for identification at the abattoir. Only healthy market pigs were selected. The gender, body weight, ear tag and tattoo information of individual pigs were recorded prior to transport.

4.3.3 Animal transport

Selected pigs were transported in weekly batches (20-25 pigs/week) from the PSC to a commercial packing plant in Saskatchewan (~2.5 hours, distance ~225 km). Pigs were fasted overnight prior to transport. The pigs were loaded around 7:15am and unloaded at about 10:45am on each transport day. Pigs were transported in a commercial livestock trailer bedded with wood shavings.

4.3.4 Unloading and infrared data collection at the plant

On arrival at the plant, the pigs were assessed visually during unloading and in lairage pens. Pigs were unloaded and moved into a single lairage pen. The time taken to unload the trailer (start and end) as well as any handling problems were recorded. The IR measurements were repeated on all pigs immediately after they entered the lairage pen. The IR measures were obtained from the pig's whole body/flank and eye region using the CG digital camera (FLIR C3). Three images from each of the body and ocular regions of individual pigs were taken in rapid succession using the CG camera. The pigs remained in the lairage pen during image collection and images were obtained at a constant distance from the pig. Digital infrared images were obtained at a distance of approximately 2 m away from the pigs'body, and 0.25 m away from the eye region.

After thermographic data collection, pigs remained in the lairage pen for approximately three hours

until being moved to slaughter. Before stunning, pigs were moved to a handling area by the handlers using shaker paddles. The pen led to a small crowd pen holding 4-5 pigs with an overhead gate controlled by ropes leading into a single file chute with V restrainer, which conveyed pigs to a manually operated head-to-heart electric stunner.

4.3.5 Temperature data extraction

The IRT images were downloaded using a FLIR software (FLIR Systems OU, Estonia). Temperatures were read from the region of interest (eyes or body/flank) by drawing a box around the digital thermographic ocular or body images. The temperature and standard deviation of the selected area were read directly from the Flir software. The ocular temperatures were read by drawing a box around the eye and its immediate surrounding (not the whole face). The average temperature within the box was recorded as ocular temperature for each image. This process was repeated for the three ocular thermographic images for each pig. The three ocular temperatures were averaged to get the mean ocular temperature. Body temperatures were read by drawing a box on the body image (skin/flank) of each pig. The average temperature and SD within this region was recorded as body temperature for each pig. The three body temperatures averaged to get the mean body temperature for each pig. If one of the three ocular or body readings varied by >1SD it was excluded from analysis.

4.3.6 Blood collection and analysis

Blood samples were collected in 10 ml plastic serum tubes (BD Vacutainer, Cat. No. 367820, Fisher Scientific) from all test pigs at exsanguination, immediately following stunning. The time of sticking and order of kill were recorded. Whole blood lactate and glucose concentrations were

measured in blood within 20 minutes of blood collection using Lactate Pro LT-1710® (Arkray Inc, Kyoto, Japan) which has been validated for veterinary use and Accu-Check glucose meter (Accu-chek guide # L9541186).The remaining blood sample was allowed to clot at room temperature for one hour. Samples were then placed on ice in an insulated container and transported to Prairie Diagnostic Services in Saskatoon for centrifugation, serum removal and storage. Sera were placed in labelled vials and stored at -80°C until analysis. Blood sample centrifugation and serum isolation were completed between 3 and 5 h after sampling. Once all samples were collected, cortisol analysis was performed on an Immulite automated analyzer (Immulite®/Immulite 1000 cortisol, Siemens Healthcare Diagnostics, Llanberis, UK) using the Immulite Cortisol Immunoassay.

4.3.7 Carcass and meat quality measurements

Pig carcasses were identified using individual tattoo numbers and placed on a separate rail in the cooler. Carcasses were split, eviscerated and chilled according to standard commercial practices. Hot carcass weight and carcass lean percentage were recorded and used to calculate the dressing yield percentage.

4.3.8 Early pH measurement

Muscle pH and temperature were evaluated on carcasses in the cooler at 1 and 3 h postmortem. Sampling was done on the 10^{th} rib of the *longissimus dorsi* (LD) muscle using a probe pH meter (Oakton pH 6+ Hand-held pH Meter, Cole-Parmer Canada Company, Montreal).

4.3.9 Meat sample collection

Meat samples from the left loin (LD) of the pigs were collected the morning of the day following slaughter. A 10 cm long section of left LD at the 10^{th} rib was collected and the individual pig ID was written on the rind side using meat marker. Loin samples were bagged and placed on ice in coolers and transported immediately to the University of Saskatchewan meat quality laboratory for analysis. In the meat laboratory, three chops of 2 cm thickness were cut from the loin muscles of each pig and analyzed for pH, color and drip loss. Another 2.5 cm cut from each meat sample was prepared for freeze loss, cooking loss and Warner-Bratzler shear force measures.

4.4. Ultimate pH (pH24) measurement

The pH24 was measured in the LD muscle at 24 h postmortem in the meat laboratory using a probe pH meter (Oakton pH 6+ Handheld pH Meter, Cole-Parmer Canada Company, Montreal).

4.4.1 Meat color measurement

Meat color was determined in the LD muscle at 24 h postmortem using a chromameter (Minolta Chromameter, CR 300, Konica Minolta, Mississauga, ON) equipped with a 25 mm aperture, 0° viewing angle and D_{65} illuminant according to the reflectance coordinates (CIE L*, a*, b*) after exposing the muscle surface to 15 minutes blooming time at 4°C.

4.4.2. Drip loss

Drip loss was assessed on the adjacent chop according to a modified EZ-Drip Loss procedure (Correa et al. 2007). Three cylindrical muscle cores were taken by a cork borer (25 mm diameter) in each slice and weighed. After weighing, the cores were placed into plastic drip loss containers

in sample racks and stored at 4 °C. Forty-eight hours after sampling, the samples were removed from their containers, carefully dabbed and weighed. Drip loss was estimated by calculating the difference between the initial and the final weight of the muscle sample in percent.

4.4.3 Meat tenderness

At 24 h postmortem, 2.5 cm thick chops were cut from the skin-on boneless loin. Chops were trimmed free of excess rind fat. The chops were vacuum packaged and stored at 4ºC for 7 days after which they were frozen at -30°C until evaluation. On the day of cooking, the frozen weight was recorded. Frozen samples (2.5cm thick chops) were thawed at 2-5°C until an internal temperature of 2-5°C was reached (approximately 24 to 36 hours prior to cooking). The thawed chops were bloomed and purge was removed using paper towel before cooking. During thawing, chop overlap and stacking were avoided to improve the consistency of the thawing process. The internal temperature of chops was verified using a hand-held thermometer (HH23A microprocessor thermometer, type J-K-T thermocouple, AO-20250-91, Cole Parmer, China) prior to cooking. The thawed chops were placed on the weighing scale and thawed weight recorded. The temperature of the thawed chops was recorded prior to cooking. Chops were cooked to a final internal temperature of 70°C using a flat grill cooking method. During cooking, the chops were turned over at 35°C. The flat grill (reversible flat grill #JXGRILL1, CGS750P2MS1, GE Appliances, Kentucky, USA) was heated to 325°F prior to cooking and the cooking time (on/off) was recorded. After cooking, the chops were held at room temperature for post-cooking temperature rise to complete. During the post-cooking temperature rise, a needle thermocouple (copper constatine thermocouple, Omega® PART# TMTSS-062G-6, Omega, Norwalk, Connecticut, USA) with diameter less than 0.02 cm and special limits of error of less than 2°C was

inserted into the geometric center of each chop and post-cooking rise was monitored with the hand-held thermometer. The maximum temperature at 2 minutes post-cooking was recorded as the final cooked internal temperature. The cooked weight (cooling time) was recorded at 50°C as post-cooking internal temperature. The cooked chops were put inside a bag and moved to a +4°C refrigerator for cooling prior to coring. Chops were chilled for 4 hours at 2 to 5°C prior to coring. During coring, squared-rectangular cores (1.6 cm in diameter) parallel to the longitudinal orientation of the muscle fibers were removed from each chop so that the shearing action would be perpendicular to the longitudinal orientation of the muscle fibers. Cores were made using a hand-held coring device. A minimum of six cores was obtained from each chop. Each core was sheared once in the center to avoid the hardening that occurs toward the outside of the sample. Shearing was done using a Warner-Bratzler shear machine (Food Technology Corp, Mecmesin serial number 21-0132-01,TMS-PRO,USA) with a WBS attachment and crosshead speed set at 200 to 250 mm/min. Final Warner-Bratzler shear force (in Newtons) was reported as the mean of all core values.

4.4.4 Statistical analysis

Pearson correlations in SAS (SAS 9.4, Statistical Analysis Systems, Cary, NC) were used to test for associations between post-transport temperatures (ocular and body), blood measures (cortisol, glucose and lactate) and carcass quality. Meat samples were classified into four categories based on color (L*), pH and drip loss. Normal pork (n=33): pH1 hr: 6.0-6.4, L*:50-54 [(Warris and Brown 1993; Correa et al. 2007); pH24 hr =5.5-5.8, DL=10.0 (Warris 2000; Correa et al. 2007; Swatland 2008)]. PSE pork (n=14): pH1 hr <6.0, pH24 hr <5.5, L*>54, DL >10% (Warris 2000; Swatland 2008). MPSE pork (n=39): Carcasses with attributes intermediate between PSE and

normal meat. PFN pork (n=14): pH24hr: 5.5-5.80, L*>50, DL<5% (Correa et al. 2007). Missing pH1 hr values (n=26) were estimated using the linear equation: y = mx + c (where y = pH 1hr, x = pH3hr, m = slope, and c = intercept). Mixed models (Proc mixed, SAS 9.4) were used to determine relationships between meat quality classifications and ocular or body temperatures and other meat quality traits. Meat quality classification was used as the fixed effect and the random factor was replicate. Significant means were separated using Tukey post-hoc test.

4.5 Results

4.5.1 Relationship between infrared temperature and physiological parameters

The descriptive statistics for post-transport temperatures and blood parameters is shown in Table 4.1. Table 4.2 shows the Pearson correlations (r, P-values and [n]) between post-transport temperatures (ocular and body) and blood parameters. There was a positive correlation (r=0.66, P<0.01) between IR ocular and body temperatures post-transport. Positive correlations were found between post-transport temperatures and blood cortisol levels (r=0.22 and 0.40 for body and ocular temperatures, respectively [P≤0.02]). There were negative correlations (r=-0.26, P=0.01) between post-transport temperatures and blood glucose levels. There was no correlation between lactate concentration and post-transport temperatures (ocular or body). Cortisol and glucose levels were negatively correlated (r = -0.23, P=0.01), and there was a positive correlation (r = 0.31, P=0.01) between glucose and lactate.

4.5.2 Relationships between infrared temperatures and meat quality

Table 4.3 shows the descriptive statistics of post-transport temperatures, carcass pH and temperatures (at 1, 3 and 24 hours), and drip loss postmortem. Average carcass pH dropped over time from 6.32 at 1h to 5.57 at 24 h postmortem. Pearson correlations (r, P-values and [n]) of post-transport temperatures, blood pH (at 1, 3 and 24 hours), and drip loss postmortem are shown in Table 4.4. There were no correlations ($P>0.05$) between post-transport temperatures and muscle pH at 1, 3 or 24 hours postmortem. Pearson correlations (r, P-values and [n]) of post-transport temperatures and carcass temperatures at 1 h, 3 h and 24 h are as shown in Table 4.5. There were significant positive correlations ($r=0.31$, $P\leq0.02$) between post-transport body temperatures and carcass temperatures at 3 h and 24 h postmortem. Table 4.6 shows the Pearson correlations (r, P-values and [n]) of post-transport temperatures, meat color and WB shear force. Positive correlations were found between post-transport temperatures and b* ($r=0.34$, $P\leq0.02$). A negative correlation ($r=-0.50$, $P\leq0.01$) was found between body temperature and WB shear force, indicating that meat tenderness increased with increasing temperatures post-transport.

Table 4.7 shows the linear regression between post-transport temperatures and muscle pH and carcass temperatures postmortem. Negative associations ($r^2=-0.3$, $P<0.001$) were found between muscle pH postmortem and post-transport temperatures. Table 4.8 shows the linear regression output between post-transport temperatures and meat color, WB shear force, drip loss and carcass quality. Meat color values (L*, a*, and b*) were positively associated ($r^2=0.16$, $P\leq0.010$) with post-transport body temperatures, while b* only was positively associated ($r^2 = 0.10$, $P=0.020$) with post-transport ocular temperature. There were negative associations ($r^2=0.70$, $P\leq0.010$) between WB shear force and IR body temperatures post-transport. Meat toughness decreased with

increasing post-transport temperatures. There were no relationships (P>0.05) between drip loss, meat marbling and post-transport temperatures. Table 4.9 shows the linear regression output between post-transport temperatures and blood parameters. Positive associations (r^2=0.15, P≤0.040) were found between post-transport temperatures and cortisol with the ocular temperature showing stronger relationship with cortisol than the body temperature. There were negative associations (r^2=-0.10, P=0.010) between IR body temperatures post-transport and blood glucose. There was no relationship between post-transport temperatures (P>0.05) and blood lactate concentration.

The LS Means for infrared temperatures and meat quality traits classification are shown in Table 4.10. Pigs that produced loins with pale soft and exudative (PSE), moderately pale soft and exudative (MPSE), pale firm and normal (PFN) meat quality had significantly (P=0.013) higher body temperatures compared to those with normal meat quality, however, ocular temperature showed no relationship with meat quality traits.

Table 4.1: Descriptive statistics for post-transport temperatures and blood parameters.

Variable	N	Mean	Std Dev.	Min.	Max.
Body temp. (°C)	120	35.90	1.32	32.00	38.70
Ocular temp. (°C)	120	36.51	0.94	33.93	38.77
Glucose (mmol/L)	119	8.00	1.50	5.50	12.80
Lactate (mmol/L)	117	14.10	5.00	2.90	23.40
Cortisol (nmol/L)	119	95.30	44.34	13.40	203.00

*Post-transport temperatures were taken at the lairage pen, blood glucose and lactate were determined using a bed-side meter immediately after slaughter, while blood cortisol was analyzed at the Prairie Diagnostic center, University of Saskatchewan. N=Number of animals, Std Dev = Standard deviation, Min. = Minimum temperature, Max. = Maximum temperature. Body temp. = body temperature, ocular temp. = ocular temperature

Table 4.2: Pearson correlation (r, p-values and [n]) of infrared temperatures with blood parameters. Significant correlations are indicated in bold.

	Body temp.	Ocular temp.	Cortisol	Glucose	Lactate
Body temp. (°C)	1.00 120	**0.66** <0.010 120	**0.22** 0.020 119	**-0.25** 0.010 119	0.06 0.510 117
Ocular temp. (°C)		1.00 120	**0.40** 0.010 119	**-0.26** 0.010 119	0.05 0.600 117
Cortisol (nmol/L)			1.00 119	**-0.23** 0.010 119	0.12 0.200 117
Glucose (nmol/L)				1.00 119	**0.31** 0.010 117

Body temp.=body temperature, ocular temp.=ocular temperature, N=Number of animals, Std Dev = Standard deviation, Min.=Minimum temperature, Max.= Maximum temperature.

Table 4.3: Descriptive statistics of carcass temperature and meat quality traits in market hogs.

Variable	N	Mean	Std Dev	Min.	Max
pH 1hr	116	6.32	2.86	5.32	6.94
pH 3hr	116	5.88	2.84	4.91	6.15
pH24 hr	116	5.57	0.22	5.01	6.53
Drip loss (%)	116	8.97	4.70	3.54	35.60
Temp. 1hr (°C)	54	37.04	3.10	23.60	41.50
Temp. 3hr (°C)	64	18.10	5.50	7.00	24.80
Temp. 24hr (°C)	118	5.30	1.20	3.40	07.60
WB Shear force (N)	99	47.11	21.74	11.92	102.30
*L**	118	54.73	4.00	38.14	65.18
a	118	9.80	1.70	5.90	14.70
b	118	5.82	1.60	0.62	10.52

*WB= Warner Bratzler shear force, *L**= light reflectance, a = greenness, and b = yellowness. N=Number of animals, Std Dev = Standard deviation, Min. = Minimum temperature, Max. = Maximum temperature. pH 1, 3 and 24 hr = Postmortem pH at 1, 3 and 24 hours respectively. Temp. 1, 3, and 24 hr = Postmortem temperatures at 1, 3 and 24 hours respectively.

Table 4.4: Pearson correlation (r, p-values and [n]) of infrared temperatures, muscle pH (at 1, 3 and 24 hours postmortem) and driploss. Significant correlations are indicated in bold.

	Ocular temp.	Body temp.	pH 1hr	pH 3hr	pH 24hr	Drip loss
Ocular temp. (°C)	1.00 120	**0.66** <0.010 120	-0.08 0.420 116	-0.07 0.480 116	-0.14 0.140 116	-0.15 0.120 116
Body temp. (°C)		1.00 120	-0.03 0.750 116	-0.01 0.940 116	-0.06 0.510 116	-0.03 0.780 116
pH_1hr			1.00	**0.42** <0.010 116	**0.22** 0.020 116	-0.01 0.930 116
pH_3hr				1.00 116	**0.23** 0.010 116	0.01 0.990 116
pH24_hr					1.00 116	-0.15 0.120 116

Body temp.= body temperature, ocular temp.= ocular temperature, pH 1, 3 and 24 hr = Postmortem pH at 1, 3 and 24 hours respectively. Temp. 1, 3, and 24 hr = Postmortem temperatures at 1, 3 and 24 hours respectively.

Table 4.5: Pearson correlation (r, p-values and [n]) of post-transport temperatures and carcass temperatures at 1 h, 3 h and 24 h. significant correlations are indicated in bold.

	Body temp.	Ocular temp.	Temp_1hr	Temp_3hr	Temp_24hr
Body temp. (°C)	1.00 (120)	**0.66** <0.010 (120)	0.10 0.510 (54)	**0.31** 0.010 (64)	**0.21** 0.020 (118)
Ocular temp. (°C)		1.00 (120)	-0.20 0.200 (54)	0.20 0.230 (64)	0.07 0.440 (118)
Temp_1hr (°C)			1.00 (54)	**0.50** 0.020 (64)	-0.20 0.220 (118)
Temp_3hr (°C)				1.00 (64)	**0.80** <0.010 (64)

Body temp.= body temperature, ocular temp.= ocular temperature, pH 1, 3 and 24 hr = Postmortem pH at 1, 3 and 24 hours respectively. Temp. 1, 3, and 24 hr = Postmortem temperatures at 1, 3 and 24 hours respectively.

Table 4.6: Pearson correlations (r, p-values and [n]) for infrared temperatures, meat color and WB shear force values. Significant correlations are indicated in bold.

	Body temp.	Ocular temp.	WB Shear force	L*	a*	b*
Body temp. (°C)	1.00 (120)	**0.66** ≤0.01 (120)	**-0.50** ≤0.01 (99)	0.10 0.3 (118)	0.20 0.07 (118)	**0.34** 0.02 (118)
Ocular temp. (°C)		1.00 (120)	**-0.41** ≤0.01 (99)	0.08 0.40 (118)	0.10 0.34 (118)	**0.22** 0.02 (118)
WB Shear force (N)			1.00 (99)	0.01 0.92 (118)	-0.04 0.63 (118)	-0.02 0.14 (118)
*L**				1.00 (118)	**0.5** ≤0.01 (118)	**0.70** ≤0.01 (118)
a					1.00 (118)	**0.82** ≤0.01 (118)

*WB= Warner Bratzler shear force, *L**= light reflectance, a = greenness, and b = yellowness. Body temp.= body temperature, ocular temp.= ocular temperature.

Table 4.7 Linear regression output between post-transport temperatures, muscle pH and carcass temperatures postmortem.

Variables*	Estimate	n	SE	R sq	T value	Pr > |t|
pH 1hr vs body temp	-0.01	109	0.10	0.41	-4.00	<0.010
pH3hr vs body temp	-0.20	109	0.10	0.30	-3.40	<0.010
pH24hr vs body temp	-0.20	109	0.10	0.52	-3.09	<0.010
Temp. pH1hr vs body temp	-1.00	54	0.40	0.31	-3.00	0.010
Temp.pH3hr vs body temp	-0.28	118	0.68	0.39	0.41	0.690
Temp.pH24hr vs body temp	0.10	118	0.08	0.01	0.78	0.072
pH 1hr vs ocular temp	-0.20	109	0.04	0.33	-4.30	<0.010
pH3hr vs ocular temp	-0.20	109	0.04	0.19	-4.30	<0.010
pH24hr vs ocular temp	-0.20	109	0.04	0.53	-4.42	<0.010
Temp. pH1hr vs ocular temp	-0.30	54	0.50	0.21	-0.61	0.550
Temp. pH3hr vs ocular temp	1.20	64	0.61	0.10	2.60	0.010
Temp. pH24hr vs ocular temp	-0.30	118	0.11	0.36	-2.30	0.030

Body temp.= body temperature, ocular temp.= ocular temperature, pH 1, 3 and 24 hr = Postmortem pH at 1, 3 and 24 hours respectively. Temp. 1, 3, and 24 hr = Postmortem temperatures at 1, 3 and 24 hours respectively, SE = standard error.

Table 4.8 Linear regression describing associations between post-transport temperatures, meat color, WB shear force, and driploss

Variables*	Estimate	n	SE	r sq	T value	Pr > |t|
a vs body temp	0.40	99	0.14	0.08	2.82	0.010
b vs body temp	0.50	99	0.13	0.16	3.60	0.001
*L** vs body temp	0.41	99	0.10	0.12	3.90	0.002
WB Shear vs body temp	-3.84	99	1.10	0.70	-3.54	0.010
Drip loss vs body temp	0.12	99	0.12	0.10	1.00	0.320
a vs ocular temp.	0.40	99	0.20	0.04	1.80	0.080
b vs ocular temp.	0.33	99	0.14	0.10	2.40	0.020
*L** vs ocular temp.	0.44	99	0.50	0.03	0.90	0.470
WB Shear vs ocular temp.	-9.10	99	2.03	0.20	-4.50	<0.010
Drip loss vs ocular temp.	-0.04	99	0.23	0.04	-0.18	0.900

* WB= Warner Bratzler shear force, *L**= light reflectance, a* = greenness, and b* = yellowness. Body temp.= body temperature, ocular temp.= ocular temperature.

Table 4.9 Linear regression describing associations between post-transport temperatures and blood parameters

Variables*	Estimate	n	SE	r sq	T value	Pr > |t|
Cortisol vs body temp	7.00	119	2.90	0.12	2.42	0.020
Glucose vs body temp	-17.50	117	0.10	0.10	-2.70	0.010
Lactate vs body temp	0.73	98	0.41	0.10	1.81	0.070
Cortisol vs ocular temp	11.10	119	3.72	0.15	2.90	0.004
Glucose vs ocular temp	0.08	119	0.13	0.10	-2.90	0.003
Lactate vs ocular temp	0.60	98	0.60	0.10	1.00	0.320

Body temp.= body temperature, ocular temp.= ocular temperature, SE = standard error.

Table 4.10: LS Means from mixed model analysis of infrared temperatures and meat quality traits by meat quality classification.

Item	Classification*					
	Normal	PSE	MPSE	PFN	SEM	P-value
N	33	14	39	14	-	-
Ocular temp. (°C)	36.39	36.76	36.56	36.65	0.35	0.564
Body temp. (°C)	35.49^{b}	36.38^{a}	36.17^{a}	35.80^{ab}	0.43	0.013
a*	9.37^{b}	10.88^{a}	9.98^{ab}	10.25^{ab}	0.39	0.032
b*	5.37^{b}	6.97^{a}	6.23^{a}	6.13^{ab}	0.35	0.004
Cook loss (%)	21.30	23.74	19.63	18.66	1.89	0.160
Shear force (N)	46.36	47.74	47.36	47.13	10.47	0.960

* Meat quality classification: Based on L*, drip loss (%) and pH at 1 and/or 24h postmortem, loins were classified as: Normal, PSE= Pale soft and exudative, MPSE= moderately pale soft and exudative, or PFN= Pale firm and non-exudative. Body temp.= body temperature, ocular temp.= ocular temperature.

^{ab} LS means within rows with different superscripts are significantly different (P <0.05; post-hoc Tukeys test).

4.6 Discussion

4.6.1 Relationship between infrared temperatures post-transport and physiological parameters.

Pig handling during loading, transport, unloading, lairage and stunning has been identified as the major ante-mortem events that could elicit negative stress-related responses (Alarcón-Rojo and Duarte-Atondo 2006) which can significantly affect animal welfare and meat quality (Becerril-Herrera et al. 2010). O'Connor et al. (2000) and Martinez-Miro et al. (2016) noted that cortisol is the main glucocorticoid that is used for measuring heat stress in pigs. In this study, there were positive correlations between infrared temperatures post-transport and blood cortisol at exsanguination. Peres et al. (2014) reported that pigs exposed to high stressful conditions prior to slaughter had high blood cortisol concentrations at sticking. It implies that IRT could be reliable for detecting temperature change related to pig welfare and transport outcomes. Based on correlation r values, there was a better agreement between ocular temperature and blood cortisol than body temperature and blood cortisol in this study, suggesting that ocular temperature could be more responsive to acute stress than body temperature in market pigs.

The negative correlations between infrared temperatures post-transport and blood glucose indicate that the pigs were exhausted as a result of the transport stress encountered. Pigs which experienced greater stress during transport (as shown by higher infrared temperatures post-transport) had lower glucose levels at slaughter, possibly due to greater activity or higher metabolic rate during transport. Owens et al. (2009) and Carvalho et al. (2014) reported that an increase in temperature could lead to an increase in the breakdown of muscle glycogen and consequently, the acidification and degradation of myofibrillar protein. The stress experienced during transport could have altered

the normal physiology of the pigs leading to increase in blood cortisol and a depletion of muscle glucose at sticking. However, there was no correlation between blood cortisol and blood lactate, possibly due to the low to moderate stress experienced by pigs in this study.

4.6.2 Relationships between infrared temperatures post-transport and meat Quality.

Meat quality involves a combination of factors such as sensory evaluations (color, texture, juiciness, taste, odor, tenderness), nutrient content (fat profile, protein content, minerals and vitamins), and technical parameters (pH, water holding capacity including drip loss) (Guerrero et al. 2013; Hocquette et al. 2015). These factors are reliable indices for catogorizing meat.

It is likely that IR measurements taken on pigs prior to slaughter will show relationships with meat quality since meat temperature (postmortem) is known to have an important impact on meat quality. This study confirmed that pig temperatures measured prior to slaughter were related to pork temperatures postmortem. The optimal temperature of pork carcasses at 1, 3 and 24 h are 35-37°C, 26-28°C, and 5-6°C respectively (Honikel 1999). Similarly, Channon et al. (2000) reported an early postmortem pork temperature (1 hr postmortem) of 35°C, which is below the mean early postmortem pork temperature of 37.4°C observed in this study. Early postmortem carcass temperature is related to the condition of an animal pre-slaughter. Sybesma and Eikelenboom (1978) observed that pigs exposed to acute stress pre-slaughter are more likely to yield high early carcass temperatures postmortem. Thompson (2002) and Hamoen et al. (2013) reported that higher carcass temperatures postmortem could lead to heat shortening in muscles which could cause meat toughness, excessive drip loss and meat paleness.

Gispert et al. (2000) and Perre et al. (2010) noted that transport stress could increase the proportions of PSE, DFD, RSE or PFN meat traits. In Canada, Fortin (1989), reported 20–90% prevalence of PSE pork, and more recently Murray (2001) and Faucitano et al. (2008) reported the occurrence of PFN and RSE (13-47%) and PSE pork (13-21%). Meat products are graded into different quality categories based on early pH, ultimate pH, color (*L**) and water-holding capacity (WHC) or drip loss (Correa et al. 2007). Pale soft and exudative pork is characterized by: pH of less than 6.0 at 1 h postmortem (Chae et al. 2007), pH below 5.5 at 24 h postmortem (Sellier and Monin 1994), and drip loss greater than 10% (Warris 2000) with a Minolta (L*) value higher than 50 postmortem. This study found significant differences between post-transport body temperatures across different meat quality categories. Body temperature had consistently stronger relationships than ocular temperature with the meat quality measures. Thus, body temperature appears to be better at predicting overall response in the body as higher significant body temperatures were found in pigs that produced loins with pale soft and exudative (PSE), moderately pale soft and exudative (MPSE), pale firm and normal (PFN) meats, whereas ocular temperature showed no relationship with meat quality traits. Pigs with PSE and MPSE meat quality had higher IR body temperatures compared to pigs that produced PFN and normal (RFN) meat traits. This indicates a positive relationship between elevated body temperatures prior to slaughter and the occurrence of PSE meat traits. The IR body temperatures at the packing plant could be used for predicting pork quality postmortem. Pigs with high IR temperature at the packing plant prior to slaughter could be identified and treated (e,g, held for additional time in lairage) to improve meat quality.

Meat color is a fundamental property for characterizing meat (Brewer et al. 2002). Good quality meat is known by its bright red color (Cornforth and Jayasingh 2004). Extrinsic factors such as

pre-slaughter stress negatively affect meat color, which could lower the quality of meat postmortem and consumer preferences. No correlation was found between L* and body or ocular temperatures, but in linear regression analyses, associations were found between body temperature and L*. In addition, stronger relationship was found between b* and body temperatures than b* and ocular temperature. The positive associations between post-transport temperatures and meat color (L* and b*) depicts the denaturation of myoglobin which could have led to drip loss and meat paleness. Wiklund et al. (2010) reported a positive correlation between meat paleness and hot carcasses. Hedrick (1965), Kerry and Ledward (1999), and Tang et al. (2013) reported that pre-slaughter stress could alter muscle metabolism and subsequently, distort ultimate pH, thus, affecting meat color and water-holding capacity in animals. This study found that the physico-chemical properties of pork carcasses could be altered by an increase in body temperature of pigs prior to slaughter.

Tenderness is one of the main attributes that buyers consider when buying meat. Meat tenderization is a complex process that involves the degradation of collagen postmortem (Veiseth et al. 2004), with reduction in the diameter of myofiber bundles (Renand et al. 2001), and changes in sarcomere length during *rigor mortis* (Rhee et al. 2004). Meat tenderness is measured using Warner-Bratzler shear force (WBS), which is the force required to cut a meat sample. A negative correlation was found between WBS and IR ocular and body temperatures post-transport, showing a decrease in meat toughness (more tender meat) with increase in IR temperatures. Moeller et al. (2010) classified pork meat into three categories: tough (>44.1N), intermediate tough (33.3-44.1N), and tender (<33.3N). In this current study, a mean WBS of 47.11 N which falls within the range of tough pork meat was reported. Meat toughness occurs when muscle pH is below 6.0 at 1 hr postmortem with an early carcass temperature greater than 35°C (Aalhus et al. 1994). This

suggests that increase in IR temperatures post-transport in pigs could increase WBS, thus, causing pork meat toughness.

4.7 Conclusion

This study indicates that using IRT to measure body temperature in pigs prior to slaughter can provide important information related to stress and meat quality. Positive associations were found between pig temperatures post-transport and blood cortisol, while negative correlations were found between temperatures post-transport and blood glucose. Relationships were also found between pig temperatures and meat quality. Pigs with higher body temperatures perimortem produced meat with PSE and MPSE quality traits. It was hypothesized that pigs with higher temperatures post-transport would show higher levels of blood cortisol and lactate, and lower levels of glucose at sticking and a greater prevalence of PSE meat traits. The significant relationships between IR body temperatures and meat quality categories shows that the body rather than the ocular region is better for measuring pig temperatures at the packing plant prior to slaughter. The application of IRT at the packing plant could help in identifying and excluding pigs with high body temperatures from pigs for slaughter to avoid slaughtering pigs that are liable to yield poor carcasses.

5.0: Overall discussion

5.1 Introduction

Infrared thermography has been used for recording temperature for health assessment in animals (Schaefer et al. 2007), welfare in horses (Fonseca et al. 2006) and sheep (Stubsjoen et al. 2009) and the response of pigs to *Actinobacillus pleuropneumonia* vaccine injection (Cook et al. 2015). Infrared thermography is preferred over invasive temperature measuring methods in animals for its accuracy and ease of use (Seshoka et al. 2013). The overall aim of this study was to evaluate the ability of IRT to predict transport stress and meat quality in market pigs.

5.2 Objectives

The primary objective of this study was to evaluate IRT as a tool for predicting transport stress and meat quality in market pigs. Specific objectives included: 1) to evaluate the ability of consumer and research grade digital infrared cameras to measure the temperature of market pigs; 2) to determine the effects of a controlled handling treatment on ocular and body temperatures in market pigs; 3) to evaluate the reliability of the less expensive consumer grade digital infrared camera for measuring temperature in market pigs pre- and post-transport; 4) to compare two regions, body and ocular, for digital infrared imaging in market pigs pre- and post-transport; 5) to determine the relationship between pig temperature and key indicators of stress in market pigs at exsanguination; and 6) to determine the relationship between pig's temperature perimortem and pork quality.

5.3 Discussion

As presented in Chapter 2, this study compared the ability of the CG and RG digital infrared

cameras for measuring ocular and body temperatures in market pigs. Results showed that pig temperatures recorded using the two cameras were significantly correlated, and that the CG camera was able to measure the increase in temperature caused by a mild handling stressor, with similar values to the RG camera. The study found that the digital infrared CG camera and the RG cameras gave similar ocular and body temperature measures, thus, supporting use of the less expensive CG camera for measuring pig temperatures by producers/packers. Correa et al. (2013; 2014) reported that on-farm handling such as moving pigs from the pen to the truck gate during loading could expose pigs to stress. In addition, the study evaluated ocular and body regions for measuring temperatures in pigs. There were effects of controlled handling treatment on ocular and body temperatures. Pig temperatures increased when pigs were handled (T2) as shown in the treatment by time interactions in the ocular measures using both cameras. This shows that even a mild handling stressor can result in a significant rise in ocular temperature in pigs. The study also showed that the ocular region may more responsive to acute stress in pigs. Kammersgaard et al. (2013) found that temperature measures from the anus, eyes, ear roots and armpits of neonatal pigs were significantly higher than the body surface temperature of other parts. This study reported that IRT is reliable for measuring temperatures, and showed evidence that the less expensive CG camera is suitable for monitoring body temperature of pigs, and could be useful in the swine industry.

Changes in body temperature can indicate that an animal is sick, in pain or stressed. The World Organization for Animal Health considers an animal to be in a state of good welfare if the animal is healthy, comfortable, well-fed and able to exhibit innate behaviors and is free from pain, fear or distress (World Organization for Animal Health 2018) at any production stage. Chapter 3 of this study evaluated market pigs using a CG digital infrared camera pre- and post-transport to

determine if temperatures measured on farm were predictive of temperatures post-transport. There were no significant correlations between IR temperatures measured on-farm (at T1 or T2) and IR temperatures measured post-transport (T3) for either the ocular or body regions, showing that on-farm temperatures were not predictive of IR temperatures post-transport in this study. There was a positive correlation between body temperatures recorded on-farm at the two time points. However, the ocular temperatures at T1 and T2 were not significantly correlated, indicating in this situation that the body region may be more reliable than the ocular region for health assessment of market pigs pre-transport/on-farm. The on-farm time 1 body temperatures (body temperatures measured after sorting, ear tagging, tattoing and weighing) were higher than the time 2 body temperatures (body temperatures measured when the pigs were calm, one day prior to transport). This confirms that animal handling on-farm such as sorting/selection in readiness for transport can increase the body temperatures of pigs. Grigor et al. (2004), Zhen et al. (2012), and Miranda et al. (2014) reported that human-animal interactions and other activities associated with transport could induce mild to severe stress in pigs. The observed positive correlations in on-farm body temperatures is evidence that IR could be useful for assessing pig welfare on-farm and prior to transport.

Ante-mortem handling procedures such as transport (Warris and Bevis 1986), housing (Brown et al. 1999) and pre-slaughter stressors (Hambrecht et al. 2004a) can negatively affect vital aspects of pork quality such as color, water holding capacity and other organoleptic characteristics. In this study, pigs with higher ocular IR temperatures post-transport had higher blood cortisol at sticking, showing that increasing ocular temperature is associated with greater activation of HPA axis.

Negative associations were found between IR ocular and body temperatures post-transport and

blood glucose, and between blood glucose and muscle pH (1, 3 hours postmortem), suggesting increased glycolysis in the muscles of warmer pigs postmortem. The elevation of blood stress biomarkers such as cortisol can alter cellular and tissue metabolism pre- and postmortem, hence, affecting the rate and extent of energy depletion and muscle pH (Romero and Butler 2007; Choi et al. 2012) which can have deleterious effects on pork quality (Lobâo 2011, Muchenje and Ndou 2011; Adzitey and Huda 2012).

The positive associations between IR body temperatures post-transport and carcass temperatures (3 and 24 hours) postmortem in this study could be responsible for a denaturation of myofibrilar proteins resulting in increased meat tenderness. Hoffman and Laubscher (2011) stated that thermal stress could result in high secretion of stress-related compounds and muscle protein denaturation postmortem. This could account for the association between more tender meat and higher body temperatures observed in Chapter 4 of this thesis. Futhermore, the positive associations between IR temperatures post-transport and meat color (L*, and b*) suggest that the myoglobin which is responsible for meat color might have been denatured due to high carcass temperature postmortem, thus, the observed meat paleness in this study, suggesting a relationship between physiological indicators of stress and carcass characteristics in pigs.

There were significant differences between IR body temperatures post-transport and carcass quality categories. Pigs with higher IR body temperatures prior to slaughter had pale soft and exudative (PSE), and moderately pale soft and exudative (MPSE) carcasses, while pigs with lower IR body temperatures post-transport had red firm and normal (RFN) carcasses postmortem, indicating poorer meat quality in pigs with higher IR body temperatures compared to pigs with lower IR body temperatures. There was no relationship between IR ocular temperatures post-

transport with meat quality categories, suggesting that IR body temperature perimortem is more suitable for predicting pork quality. This result agreed with the result of Faucitano and Geverink (2008) who reported that poor pre-slaughter handling practices (eg. feed withdrawal, loading/unloading, transport, mixing, and human-animal interactions) could result to losses in carcass value such as pale, soft, exudative and dark, firm, dry pork. This study shows that there is a relationship between IR body temperatures collected on market pigs at the packing plant and physiological and meat quality traits postmortem. The use of IRT on-farm and in lairage could help to identify pigs that are liable to transport loss and poor quality meat at slaughter.

5.4 Recommendations and future research

Automation of digital thermography at a commercial level could help to identify thermally stressed pigs in the barn pre-transport and in the lairage pen prior to slaughter, thus mitigating the transport of febrile pigs and reducing the occurrence of undesirable meat traits postmortem. A small sample size and low ambient temperature, resulting in low-moderate heat stress, were some of the limitations in this study. If a higher level of heat stress had been imposed then relationships between on-farm and post-transport temperatures may have been found. The accuracy, repeatability and reproducibility of the digital infrared cameras were not considered in this study and could be evaluated in future studies. Automation of IRT is recommended for data capturing on-farm and at the packing plant in real time to improve pig welfare and meat quality. Based on the results in Chapter 4, the body region showed greater potential than the eye region for predicting pork quality. Future work should focus on the evaluation of IRT under differing environmental conditions and improving the calibration of IR cameras using black box or similar control for improved data collection.

5.5 Conclusions

This study validated consumer and research grade digital infrared cameras for measuring ocular and body temperatures in market pigs. The less expensive CG camera measured similar temperatures compared to the RG camera, and could be beneficial to the pork industry for monitoring pig temperatures. Strong positive correlations were found between ocular and body temperatures at the same time point. There were effects of controlled handling treatment on the ocular and body temperature of pigs. The IRT was able to measure a change in temperatures resulting from a mild stressor, suggesting it could be used as a non-invasive tool to measure on-farm handling stress in pigs. The body temperature showed wider range of temperatures and produced a better correlation between cameras.

The CG camera showed promise for on-farm screening of market pigs prior to transport. There were positive correlations between body temperatures measured on-farm at two time points, indicating that IRT could measure temperature change in market pigs on-farm and prior to transport. There were positive correlations between times 1 and 2 body temperatures on-farm, showing that body rather than the ocular region could give a better prediction of temperature change in market pigs on-farm/pre-transport. However, on-farm IR temperatures (times 1 and 2 IR temperatures) were not predictive of IR temperatures post-transport. The prevailing low ambient temperature conditions within the period of this study could account for the lack of association between on-farm and post-transport temperature measures. The average T1 temperatures were higher than T2, but similar to T3 temperature measures. This observation is in agreement with the results from Chapter 2 indicating that handling treatment could increase body temperature of pigs on-farm. The positive associations between pig IR temperatures post-transport

and blood cortisol and negative correlations with the blood glucose shows that pigs with higher IR temperatures had a greater stress response. Higher IR body temperatures and higher b* values were found in pigs with PSE and MPSE compared to pigs with RFN meat traits, an indication that IR body temperatures collected prior to slaughter at the packing plant could predict meat quality in pigs. The significant relationships between IR body temperatures and meat quality categories shows that the body rather than the ocular region is better for measuring pig temperatures for the purpose of predicting meat quality outcomes. This finding is in agreement with the results from Chapters 2 and 3 of this thesis where body temperatures showed better correlations than ocular temperatures. The application of IRT on-farm could help in identifying febrile pigs prior to transport to minimize in-transit losses, and non-ambulatory pigs post-transport. In addition, IRT could help to identify pigs with high body temperatures at the packing plant to avoid slaughtering pigs that are liable to yield poor carcasses. The challenges faced in this study were small sample size and low ambient conditions during the period of study. Further testing with a larger sample size and under more varied ambient conditions is recommended to better quantify the ability of IRT for predicting heat stress and meat quality in pigs.

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