Effect of pre-slaughter stress of pigs on the levels of cortisol, creatine kinase and their subsequent relationship with pork quality

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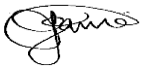
Declaration

Apart from the assistance received that has been reported in the acknowledgements, references and in the appropriate places in the text, this dissertation represents the original work of the author. No part of this dissertation has been presented for any other degree at any other University.

Jama N.…  ……..…..… Date ……2014

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Abstract

Effect of pre-slaughter stress of pigs on the levels of cortisol, creatine kinase and their subsequent relationship with pork quality

The study was conducted to determine the effect of pre-slaughter stress of pigs on the levels of cortisol, creatine kinase (CK) and their subsequent relationship with pork quality. From our knowledge, there is paucity of information on transportation, lairage duration and sex effects on saliva cortisol and its relation with serum cortisol, urine cortisol and other meat quality traits. Stage one of the study assessed the effect of sex and time to slaughter (transport and lairage duration) on the levels of cortisol and CK in crossbred pigs. The second stage of the study evaluated the effect of sex and lairage duration on pork quality. The trials comprised of 60 22-weeks old commercial crossbred pigs [(30 boars vs. 30 gilts) with an average body weight of 76.9kg for gilts and 78.3kg for boars. Thirty pigs (15 Boars vs. 15 Gilts) were slaughtered on arrival after a 120km road journey and the other 30, after travelling the same road and distance, were slaughtered after having been in the lairage for 20 hours. Saliva samples were obtained during three time periods: between 07:00-10:00 on the day before the journey, on arrival at the abattoir and after lairage. Levels of cortisol in serum and urine and CK in serum samples collected at slaughter were determined. The samples (saliva and serum) were centrifuged at 20^{o}C for 10 min at 3550 x g and stored at -20^{o}C in separate vials until analysis. Pork quality was assessed using muscle pH, colour, thawing and cooking losses and Warner-Bratzler Shear Force. Correlations between cortisol levels in saliva, serum and urine and meat quality were assessed.

The effect of sex and time to slaughter on saliva cortisol was significant. Statistical analysis showed significant interaction of sex by time to slaughter on serum cortisol. It was only the

effect of sex that demonstrated higher (P <0.05) serum creatine kinase levels in gilts. Likewise, urine cortisol was influenced (P <0.05) only by time to slaughter. Saliva cortisol after transportation (r = 0.52) was correlated (P <0.05) with urine cortisol. The study suggests that there are greater responses of the Hypothalamic-pituitary-adrenal axis following time to slaughter (transport and lairage duration) between gilts and boars. This can be used in measuring cortisol in saliva, serum and urine, and serum creatine kinase to determine stress levels in pigs.

Besides the lightness (L*) colour value which was significantly lower (P <0.05) in meat from gilts than from boars, the results of this study showed no interaction or individual effect (sex or lairage duration) on pH and other colour measurements. Meat cooking loss was affected (P <0.05) by gender x lairage interaction. Effect of lairage duration (as an individual effect) on the measured meat quality traits was not significant (P <0.05). Correlation analysis showed significant (P <0.05) negative relation between baseline saliva cortisol (r = -0.40) and the pork L* value. Saliva cortisol after lairage duration (r = -0.38 and r = 0.38) was correlated (P <0.05) with pH_{45} and pork a* value, respectively. Serum cortisol (r = -0.35) was negatively correlated (P <0.05) with pH_{45}. Moreover, there were negative correlations (P <0.05) between saliva cortisol after transportation (r = -0.35), saliva cortisol after lairage duration (r = -0.44), serum cortisol (r = -0.40) and meat cooking loss. The study suggests that cortisol in saliva and serum can be used to determine effect of time to slaughter (transport and lairage duration) on meat quality traits from gilts and boars.

Keywords: Boars; gilts; saliva cortisol; serum cortisol; serum creatine kinase; urine cortisol

Dedication

This dissertation is dedicated to my family, particularly to my Mother Mampisi, my aunt Zukiswa, and my siblings Vuyo, Vuyokazi, Mgqwaliso, Ntombi and Sixolile, and last but not least Sips Jama ka Sjadu; without their love and support this journey would not have been possible.

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Abbreviations

CIE a* - Commission Internationale de l'Eclairage redness

CIE b* - Commission Internationale de l'Eclairage yellowness

CIE L* - Commission Internationale de l'Eclairage lightness

CK - Creatine Kinase

Cklos - Cooking loss

HPA - Hypothalamic-pituitary-adrenal axis

LD - *Longissimus dorsi*

pH_{45} - Initial pH at 45 minutes

pH_u - Ultimate pH at 24 hours

Tlos - Thawing loss

WBSF (N) – Warner-Bratzler Shear Force in Newtons

PSE - Pale, soft, exudative

DFD - Dark, firm, dry

PROC GLM (SAS) - Generalised linear model procedure of Statistical Analysis System

Chapter 1: General Introduction

1.1. **Introduction**

During the conversion of pig muscle to pork, there is an array of likely stressors (*viz*. human interaction and social settings) which can adversely impair pig welfare and consequently their meat quality (Maria *et al*., 2005; Delezie *et al*., 2007; Adenkola *et al*., 2011). Stress can modulate natal processes such as immunocompetence which may express poor pig welfare (Beerda *et al*., 1999; Morrow, 2002; de Fontaine *et al*., 2006). Pig welfare is a state of well-being in which pig suffering is avoided whilst its basic needs (*viz*. food, water and shelter) are met (Spedding, 2000; Muchenje *et al*., 2009a; Muchenje and Ndou, 2011). Exposure of pigs to stress can result in various endocrine responses and thereby disrupting glucocorticoids and creatine kinase's normal activities by mobilisation and repartitioning of energy (Möstl and Palme, 2002; Romero and Butler, 2007; Macbeth *et al*., 2010).

Though costly and strenuous, conversion of pig to pork is a necessary part of today's pig production system (Leman, 2012). Costs not only include the out-of-pocket cash for transportation services but also the losses incurred from physiological exercises which may lead to low quality products (Fowler, 1961). Due to poor infrastructure and operational techniques in communal based production units, disturbance of the pig's welfare is always a possibility (Morgan, 1995; Ljungberg *et al*., 2007; Muchenje *et al*., 2009b). Thus, the desired optimal, consistent and uniform carcass can be severely impaired, as also the decisive factors for measuring pork eating quality (Faucitano and Schaefer, 2008). The stress encountered by pigs during any human-interaction can trigger biochemical processes which in turn cause secretion of hormones and enzymes and initiate their action on the protein building blocks of meat (Chambers *et al*., 2001; Cussen and Garces, 2008). Such activities can be noticed by increased heart rate and body temperature which can intensify the effects of muscular activity

on *ante-mortem* and *post-mortem* metabolism (Roldan-Santiago *et al*., 2013). This can be attributed to the flow of reactions stirred by the sympathetic nervous system (rapid conversion of proteins and lipids to glycogen) to generate energy to compensate for the resulting homeostatic imbalance (Berg, 2001; Choi *et al*., 2012). Subsequently, this can result in high pre-slaughter lactate levels measured as low *post-mortem* muscle pH which can cause weaker pigs that are prone to stress, to die during transit, and decrease consumer demand following high drip losses exhibited by the meat (Hambrecht *et al*., 2004; Grandin, 2007).

Meat acceptance and purchasing by consumers is influenced amongst others, by the earlier consumption experience, price or cultural values (Xazela *et al*., 2011; Vimiso *et al*., 2012). Consumers also judge meat according to their own sensory individuality which is determined amongst others, by the meat's ultimate pH (pH_u) (Ngambu *et al*., 2011; Gajana *et al*., 2013). However, meat pH can be radically influenced when stress-related compounds (*viz.* cortisol and creatine kinase) surge into the pig's body just prior to slaughter, thus affecting the product quality. Research has been conducted on pre-slaughter animal handling effects on meat pH (Mach *et al*., 2008), bruises and creatine kinase (CK) in sheep (Chulayo and Muchenje, 2013), and other meat quality traits (Muchenje *et al*., 2008; Sabuncuoglu *et al*., 2011). However, information on cortisol and CK activities in crossbred pigs slaughtered under conventional conditions without stressing during sampling is still lacking. Therefore, there is a need to consider sample collection without stressing the pig as other methods do and complicate the results, be unethical, and/ or time-consuming (Bodnariu, 2008; Seshoka *et al*., 2013). In addition to being fairly easy to collect and being a non-invasive method to establish any stress-induced response, saliva cortisol reflect only the bioactive fraction over a period of time prior to sampling, and is not bound to cortisol-binding globulin or other proteins (Beerda *et al*., 1996; Gozansky *et al*., 2005; Mohamed *et al*., 2012).

1.2. **Justification**

Cortisol and creatine kinase (CK) are key to improve fitness of the pig during its interaction with the surroundings (Aziz, 2004). However, sudden pig exposure to stressors may threaten its ability to make the basic needed adjustments for adaptation, and thus speed-up secretion of cortisol and CK by the endocrine system (Athayde *et al*., 2013). High secretion of stress-related compounds can increase lactate production through their glycogenolysis effect and negatively impact on muscle cell function and bone metabolism (Möstl and Palme, 2002; Geverink *et al*., 2012). The secretions can remain in the carcass for an extended period before they decline to their basal level through a negative feedback system. These can affect pork quality through muscle acidification (measured as pH_u), and use of such tainted pork can cause unwanted medical conditions and diseases (*viz*. metabolic dysfunction and impotence) (Choi *et al*., 2012). Pork pH_u which can affect important pork quality traits (*viz*. colour, water-holding capacity and shelf-life) is important in meat science and technology. Pork quality is affected amongst others, by the immediate pre-slaughter management and the unfriendly surroundings starting from the farm where pigs are reared (Kelling, 2008). Studies pertaining to glucocorticoids, and CK determination have traditionally relied on urine samples (Muchenje *et al*., 2009b), ignoring concentrations in saliva. Hence, this study focused on saliva sampling, starting from when pigs are loaded since consumers are becoming more concerned with the origin of meat as well as the whole value chain (Muchenje *et al*., 2008). Human-animal interaction during conversion ((un)loading, transport, lairage, etc.) of pig into pork is a major factor which can stir biochemical processes, disturb baseline levels of stress-related indicators, and thus affect the meat pH_u and quality (Muchenje *et al*., 2009a; Chulayo *et al*., 2012). Therefore, this study will provide information on the effect that transportation, lairage duration and sex might have on pork quality in relation to cortisol and creatine kinase in crossbred pigs.

1.3. Main objective

The main objective of the study was to determine the effect of pre-slaughter stress on the levels of cortisol, creatine kinase and their subsequent effect on the quality of pork from crossbred gilts and boars.

1.3.1. Specific objectives

- To determine the effect of sex and time to slaughter (transport and lairage duration) on the levels of cortisol in saliva, serum and urine, and serum creatine kinase in crossbred pigs.
- To determine the effect of sex and lairage duration on pork quality.

1.3.2. Hypothesis

Null hypothesis were that:

- ✓ Sex and time to slaughter (transport and lairage duration) has no effect on the levels of cortisol and creatine kinase in crossbred pigs destined for slaughter.
- ✓ Sex and lairage duration has no effect on pork quality.

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Chapter 2: Literature Review

2.1. **Introduction**

In South Africa, the relative negative image of pork consumption per *capita* since the early 70s from 3.5kg to 2.7kg in 2006 was associated with some economic factors and health problems (Oyewumi and Jooste, 2006). However, due to pork quality, taste and nutrition, the probability of pork attractiveness may increase from 0.22 to 0.43 (approximately 21%) if pig welfare is good (Bender, 1992; Luppnow, 2007; Okrouhla *et al*., 2010). Pig welfare is a complex process that starts from the farm during reproduction to the market when pork is graded free of contaminates, reliable and has desired consumption attributes and therefore is free of worries to the consumers (Yeung and Morris, 2001). Genetics, season, as well as handling at the farm and pre-slaughter handling of the pigs are very important aspects which can influence secretion of stress-related indicators (creatine kinase, cortisol and catecholamines) and thus, are responsible for the development of aberrant meat quality (Vermeulen *et al*., 2014).

Consumers are becoming increasingly aware of welfare issues and if these are not adhered to, it may decrease acceptance of pork by consumers. Creatine kinase (CK) and cortisol are muscle-specific enzymes and hormones, respectively which may indicate pig suffering especially, from physical exercise (Grigor *et al*., 2004). Their secretion together with catecholamines can be enough to aggravate the impairment of energy (carbohydrates, lipids and proteins) metabolism by muscles to counteract survival (Grandin, 2000; Ndlovu *et al*., 2007). In addition, the process of transporting animals to the abattoir can indirectly influence the decision and acceptance of pork purchase by directly affecting the quality and nutritional value of the end product. For pork to be termed “good quality”, it must meet the consumer’s expectation by having a sensory appeal that includes a uniform appearance throughout the

entire cut, be juicy, tender and must have an appetising flavour (Kerry *et al*., 2002; Radder and Roux, 2005; Muchenje *et al*., 2008). These quality attributes are physiologically dependent upon the ultimate muscle pH (pH_u), which is determined by the glycogen levels in the muscles (Morrow, 2002). The levels of glycogen (muscle carbohydrates) can reflect the amount and type of pre-slaughter stress experienced by the pigs and the way they respond to the stressor (Morrow, 2002). The objective of this study was therefore, to determine the effect of pre-slaughter stress on the levels of cortisol and creatine kinase and their subsequent relationship with pork quality under conventional conditions.

2.2 **Pre-Slaughter Stress**

Stress is a biological response elicited by pigs when physical or mental threat of homeostasis is perceived (Moberg and Mench, 2000; Kim *et al*., 2011; Lobâo, 2011). Such threat can elevate the heartbeat and fuel high secretions of enzymes and hormones to the circulatory system and thus, decrease the consumption quality expected (Romero and Butler, 2007; Correa *et al*., 2010; Lobâo, 2011; Chulayo *et al*., 2012). According to Grandin (1997), laboratory trained pigs can respond to deviations in their daily routine (which they experience as being stressful) with a rise in heart rate and blood pressure which in turn can arouse secretion of creatine kinase, cortisol, dopamine and catecholamines.

2.3 **Animal Stress-related Chemical Compounds (Cortisol and Creatine Kinase)**

2.3.1 **Creatine Kinase**

Creatine kinase is an index of physical stress which responds rather rapidly, and has a half-life of about five hours in pigs (Warriss *et al*., 1998). Its classic effect is to regenerate

Adenosine Tri-phosphate to maintain energy homeostasis. Adenosine Tri-phosphate (ATP) is the primary energy source in living organisms for energy demanding processes in cardiac and skeletal muscles, brain, retina and primitive-type spermatozoa (Chulayo and Muchenje, 2013).

2.3.2 **Cortisol**

Cortisol (steroid hormone) is an index of psychological stress, which is produced in the cortex of the adrenal glands located on top of each kidney (Warriss *et al*., 1998; Choi *et al*., 2012). Its classic effect is to select the right type and amount of substrate (carbohydrate, fat or protein) needed to increase the blood sugars available to tissues involved in responding to a stressor (Romero and Butler, 2007; Qasim and Nabi, 2014).

Already at five days of age, levels of stress-related indicators in pig breeds may be equivalent to that of a fully grown pig (adult levels) (Sutherland *et al*., 2005). Poor operational techniques, unsociable settings and or sampling method used to asses stress may all lead to increased central stimulation of the hypothalamic-pituitary-adrenal axis, resulting in elevated secretion of stress-related indicators which may or may not be sustained (Beerda *et al*., 1997; 1999). As described, uninviting environments during conversion of pig to pork can overtax the hypothalamus-pituitary-adrenal axis and threaten the pig's ability to cope to stress by demanding energy mobilisation (Kelling, 2008). High elevations of stress related indicators in the aforesaid body structures can alter the effects of some cellular and tissue activity on *ante-mortem* and *post-mortem* metabolism and thus, affect the rate and extent of energy depletion and muscle pH_u (Romero and Butler, 2007; Choi *et al*., 2012). This can significantly affect the pork's physico-chemical attributes (*viz*. pale soft exudates, red soft and exudative or dark firm dry, decreased shelf-life, tenderness, and high cooking losses) due to alteration in the rate and extent of *post-mortem* muscle pH decrease through lactic acid

production (Hambrecht *et al*., 2005a; Kerner, 2005; Lonergan, 2008; Muchenje *et al*., 2009a; Lobâo, 2011; Muchenje and Ndou, 2011; Adzitey and Huda, 2012).

2.4. **Lactic acid**

Lactic acid is a by-product of glycolysis that results from the effect of hypothalamus to the adrenal via the pituitary, and its production is directly proportional to the glycogen levels in the muscle prior to slaughter (Van Laack *et al*., 2000; Ronzio, 2003). It is arguably the largest determinant of other pork eating qualities (Kerry *et al*., 2002; Hambrecht *et al*., 2005b; Bee *et al*., 2006; Ndou *et al*., 2011). This was supported by Hambrecht *et al*. (2004) who established that muscle glycogen has a direct effect on drip loss and pork colour, with low levels of glycogen *ante-mortem* being associated with lower drip losses and darker pork colour. Since pork products are also significant sources of polyunsaturated fatty acids in the human diet (Okrouhla *et al*., 2010), an important factor in reducing the risk of cardiovascular malady (Kadim *et al*., 2006); any attempts in creating a stress-free environment to balance glycogen homeostasis prior to slaughter can be sound; both for the industry and nutrition wise.

2.5. **Factors Affecting Pork Quality**

2.5.1. **Effect of Feeding Practices on Pork Quality**

Pre-slaughter feed deprivation can prove to be a system that producers can apply to save costs as it is frequently argued that the feed will not be converted to meat prior to slaughter; however, that can disrupt the pig's homeostasis and thus, influence its behaviour and physiology (Becker *et al*., 1989; Ferguson and Warner, 2008; Salmi *et al*., 2011). This can be due to the mobilisation of the pig's body nutrient stores to maintain life processes. Such mobilisation can impair concentration of stress-related chemical compounds (*viz*. cortisol and

creatine kinase) and lead to negative alterations in the *post-mortem* muscle biochemistry and consequently, affect the muscle pH (Zhang *et al*., 2008; Salmi *et al*., 2011). Admittedly; energy consumption is gene dependent, such *post-mortem* muscle alterations can result in tissue dehydration and catabolism trying to negate energy imbalance (Leheska *et al*., 2002; Ferguson and Warner, 2008). According to Schaefer *et al*. (2001), lack of freedom from feed is a physiological stress which can trigger rapid CK and epinephrine activities such as the conversion of creatine and adenosine triphosphate by the former and lipolysis by the later. Such activities can thus change muscle's lipid composition and elevate levels of nitrogen in the plasma. Catabolic (breaking down) functions at rising levels of and high levels of energy sources can compromise the synthesis of biochemical catalysts such as catecholamines, cortisols, CK and peptides. Consequently, the pig can become less able to function normally and thus becomes incapable of maintaining its energy homeostasis (Schaefer *et al*., 2001). This can alter the *post-mortem* muscle acidity (pH_u) in favour of spoilage microbes (Leheska *et al*., 2002). Such microbes can get access to the decreased acidity and work on protein units to generate undesired volatile metabolites thereby producing foul smells, unpleasant tastes (Lawrie, 1966; Pethick *et al*., 1995; Davies and Board, 1998), dark, firm dry cuts and lessen the pork's shelf-life (Warriss, 2003).

Though access to feeding, especially tryptophan-rich diets before moving from the farm unit can reduce response to stress through its role in regulation of serotonin and thus, low cortisol production (Guzik *et al*., 2006), it can increase chances of gut fill contamination and/ or decrease the *post-mortem* space availability within the myofibrillar proteins to hold fluids (Adzitey, 2011). The decreased myofibrillar space can be due to the balanced net charge in the side chains of protein units. High (greater than 40 - 50 mol glucose per kg muscle) *post-mortem* energy levels can result in pronounced muscle acidity (pH_u <5.6) (Pethick *et al*., 1995; Muchenje *et al*., 2009a). At this point, also called the iso-electric point, protein units

have a neutral net charge. Such pH levels in conjunction with high muscle temperatures (39.86^{o}C) can result in reduced diameter of myofibrils and myofibers, leading to a rise in water content as drip and reduced solubility of proteins. This can lead to a decreased muscular functionality such as retention of natural fluids and light reflection (Hagen *et al*., 2008; Jama *et al*., 2008; Muchenje *et al*., 2009a; Muchenje and Ndou, 2011). According to Hagen *et al*. (2008), at low muscle pH_u (< 5.6), degradation of myofibrillar proteins can result in leaking of body fluids from the muscle tissue, especially during processing. This can be due to an increased activation and secretion of the cathepsin pro-enzymes stored within the lysosomes, increased transcription, reduced amounts of inhibitors in the muscle, or a combination of these factors (Hagen *et al*., 2008). Therefore, since pork is usually paid for according to its weight, decreased muscular activities can have negative economic implications to the industry because it can substantially reduce both the weight of pork and the water soluble nutrients which are vital for human consumption (Muchenje and Ndou, 2011; Meskinyte-Kausiliene, *et al*., 2012).

2.5.2. **Pre-Slaughter Handling of Pigs**

Pre-slaughter pig handling involves all the activities and processes pigs are subjected to before slaughtering (Yiu *et al*., 2001). Such activities begins at the farm unit and encompasses gathering pigs to a loading unit, loading of the pigs, transport and offloading at the abattoir where they are held in the lairage before stunning and subsequently sticking (Adzitey *et al*., 2011). Any severe activity during handling can result in changes in the metabolites of the muscle which in turn can be responsible for the differences in the ultimate properties of pork (Aziz, 2004). The nature of the changes depends on such factors as the amount and the type of stress placed on the pig and the level of the pig's stress resistance (Morrow, 2002).

Changes in muscle metabolites can be due to undue or excessive noise (Meat and Livestock Australia, 2000), or most importantly by forceful contacts such as electric goads, or hitting and handling or when taking blood samples (Grandin, 2000; Kirschbaum and Hellhammer, 2000; Muchenje and Ndou, 2011). Such forceful activities can result in serious injuries and bruises especially in pigs from poor basic management because they are likely to react imperfectly (Grandin, 2007; Gregory, 2008; Muchenje and Ndou, 2011). Bruise is caused by the escaping of blood from its ruptured vessels due to rough activities such as stick or stone hitting, fighting or falling or running against a fixed object (Warriss, 2000). Bruising can stimulate the release of enzymes and hormones (or their metabolites) into the fluid streams which in turn, can arouse the pig to exhaust rapidly its energy stores prior to slaughtering (Lawrie and Lawrie, 1998; Chulayo *et al*., 2012).

In addition to excessive stress losses, pigs can arrive at the slaughterhouse either dead or crippled from injuries received during rough or strange handling or even get strenuous exercises at the slaughterhouse. The increase in number of injuries such as bone breakage can contribute to the increase of metabolic exhaustion (Berg, 2001). This can stimulate the secretions of the chemical compounds (*viz*. cortisol and creatine kinase) into the fluid system (Elrom, 2001). Such stress-related indicators can exacerbate the muscle function and hasten the conversion of creatine to phosphocreatine for energy storage in tissues as a response to the physical stress (Kannan *et al*., 2002). This process may lead to a reduced production of *post-mortem* lactate levels in addition to the metabolic processes which could have started from the first few minutes of human contact (Chulayo *et al*., 2012). Low lactate production can then lead to a reduced ultimate *post-mortem* acidity which can result in dark, firm, dry cuts, greater water holding capacity and unpredictable tenderness (Muchenje *et al*., 2008). Such low ultimate *post-mortem* acidity (pH >5.8) can be supportive to pathogen growth and

result in poor meat processing due to insufficiency in controlling the *post-mortem* growth of *Pseudomonas* spp. thus, reducing the shelf-life of the pork (Varnam and Sutherland, 1995; Muchenje and Ndou, 2011; Adzitey and Huda, 2012). *Pseudomonas* is a bacteria that can depreciate the quality of pork due to its prowess to thrive in high pH (> 5.8) environments where it can degrade amino acids producing ammonia and off-odours (Varnam and Sutherland, 1995). The effect of bruising and injury by broken capillaries which pass through the subcutaneous fat can increase the activity of cortisol and the enzyme creatine kinase thereby exhausting the muscle's energy sources (Lawrie and Lawrie, 1998; Partida *et al*., 2006). In addition to poor appeal due to blood splashes or speckles to both processors and consumers, the aforementioned physical injuries can reduce market profit and increase expenses for labour that have to do the trimming of the bruised or injured muscle parts (Morgan, 1995; Muchenje and Ndou, 2011).

2.5.3. **Transportation of pigs**

Pig transportation to the abattoir is by its nature an inevitable, unfamiliar and threatening event that can affect pigs either physically or physiologically depending on the transportation mode, technique, duration, environment and the pig's own inherent susceptibility to stress (Aziz, 2004; Adenkola *et al.,* 2009; Leman, 2012). The greatest cause for concern either by hoof or vehicle mode is the lesions such as bruise, scratches, and bites which can reduce carcass value and impair welfare (Grandin, 2000; Mounier *et al*., 2006) especially for distances shorter than 50 km or longer than 100 km (Kerner, 2005), as shown below in Table 2.1.

Table 2. 1: Effect of distance (km) on pork pH

	0 KM	1-50 KM	51-100KM	101-200KM	201-300KM
Number of Carcasses	155	1149	928	201	394
pH_{45}	6,05	6,04	6,24	6,22	6,22
PSE %	8,4	12,9	2,5	4,0	3,0
DFD%	-	-	0,5	0,5	0,5

Adapted from Kerner (2005)

Extended overland drives (more than three hours) may be beneficial in allowing recovery from previous human-animal interaction; however, such chance to restructure for social order can increase pig depression, especially with poor stocking density and inclement environmental conditions (Lesser, 1993; Schaefer *et al*., 2001; Aziz, 2004; Gajana *et al*., 2013). Such depression can trigger secretion of CK, cortisol and catecholamines into the fluid stream (Muchenje *et al*., 2009b). This can result in lower-than-normal muscle energy stores prior- and post-slaughtering resulting in insufficient lactate and hydrogen compounds. Such an insufficiency can consequently lead to higher-than-normal *post-mortem* ultimate muscle pH (pH_u >5.8) and therefore, result in economic losses to farmers and processors not only due to dark colour perception but also due to the low keeping characteristics of the pork (Becker *et al*., 1989; MLA, 2000; Wulf *et al*., 2002; Muchenje *et al*., 2008; McEwen and Mandell, 2011).

According to Gerritzen *et al*. (2013), livestock transportation is a difficult subject to measure because of the many variables involved (*viz*. novelty, handling, vibration, temperature, sex,

stocking density, *et cetera*). Stocking density (expressed as mass of pig per meter square) is the amount of floor space provided per pig within a vehicle (Moore, 2010). It is an important part of pig welfare assessment as it can impede the pig from performing normal functions such as resting, elimination and interacting with its surroundings (Dean, 2005; Moore, 2010). Gade and Christensen (1998) supported this by stating that as a law, all pigs should as a minimum be able to stand and lie down naturally and that can be achieved amongst others, by appropriate stocking density (0.425 m^2 per 100 kg pig), pig's physical condition or transport duration. However, the above mentioned stocking density may not be appropriate for short transport durations as it can result in more aggressiveness and mounting behaviours compared with 0.35 m^2 per 100 kg pig (Gade and Christensen, 1998; Maria *et al*. 2005). As a result, it may be hard for pigs to adapt in the new environment and recover from the stress of farm handling, and consequently that can stir-up rapid secretion of stress-related indicators (cortisol and CK) prior to slaughter (Hoffman and Fisher, 2010; Choi *et al*., 2012). Such secretions can as a result increase the risk of intra-muscular acidosis and PSE through their glycogenolysis effect in the absence of oxygen post-slaughtering (van Laack *et al*., 2000; Hoffman and Fisher, 2010). High responses to *ante-mortem* stress (travel) which is specifically related to muscle pH tend to decrease meat and carcass quality including body weight, and increase drip loss and reflectance (L*) values (Hall and Bradshaw, 1998). Rapid pH fall (from near neutrality) due to lactate and hydrogen ions accretion while muscle temperature is relatively high can denature sarcoplasmic (myoglobin) and myofibrillar (myosin) proteins (Hoffman and Fisher, 2010; Hoffman and Laubscher, 2011). This can reduce their solubility and ability to retain natural water and cause them to reflect rather than absorb light, thus pale, soft and exudative product (Carlin *et al*., 2006; Lonergan, 2008; Muchenje and Ndou, 2011). In addition to that, the potential of the proteolytic enzymes

(calpain and cathepsins) to affect proteolysis for pork tenderisation can be reduced at low muscle pH and, so consumption quality (Hall and Bradshaw, 1998; Daszkiewicz *et al*., 2009).

On the other hand, high stocking densities (less than 0.42 m^2 per 100 kg pig) can suppress or displace one or more of the pig's normal activities and cause aberrant behaviour and physiological changes which can lead to poor welfare and economic performance (Gade and Christensen, 1998; Dean, 2005). This was supported by Gregory and Grandin (2007) who stated that high stocking densities (<0.4 m^2 /100 kg) by road transport can precipitate mortalities and colour defects especially under high temperature conditions (in excess of 15^oC) and by pig placement in the vehicle. This has been attributed to the suppressed activities (*viz*. desire to rest) to counteract the stressor in conjunction to high secretion of cortisol, CK and catecholamines in animals in the front compartment of the same vehicle (Richardson, 2002; Warriss, 2010; Gerritzen *et al*., 2013). High secretion of the above compounds can stimulate constriction of blood vessels for some time which can result in pigs not being able to effectively dissipate heat (Romero and Butler, 2007; Gerritzen *et al*., 2013). Such heat can then accumulate inside the pig's body in conjunction with road dust and exhaust fumes. As a result, this causes discomfort which can lead to mortality on the way to the slaughter unit (death on arrival) (Berg, 2001). This was supported by Muchenje *et al*. (2009a) who found that physiological stress caused by adverse weather conditions increases the catecholamine hormones, especially when pigs have been deprived of feed before transportation.

2.5.4. **Lairage duration**

Lairage is a place of rest at the slaughter unit for pigs to recover from the previously encountered stress during handling and transportation (Aziz, 2004; Hambrecht *et al*., 2005a; Ferguson and Warner, 2008). It is not supposed to be a stressful place by itself; however, laxity by stakeholders such as overstocking, poor ventilation, and high noise levels, extended durations, sorting and weighing, and moving pigs to slaughter pens can become stressful (Kerner, 2005). Such events can cause skin lesions or muscle damages which can lead to rapid utilisation of energy sources and consequently alterations in *post-mortem* ultimate pH (Hambrecht *et al*., 2005a). According to Gispert *et al*. (2000) and Warriss (2003), extended waiting periods of more than three hours is a long-term stress that cannot only decrease carcass weight and carcass temperature, but also increase muscle fatigue and reduce the muscle sugar concentration at slaughter, producing pork colour defects. According to Morgan (1995), these phenomena tend to occur particularly in overnight pigs deprived of feed.

Including interaction between sexes and reduced feed intake, long lairage times (> 3 hours) can increase the utilisation and synthesis of enzymes and hormones (CK and cortisol respectively). However, due to the deprived feeding, components such as tyrosine for hormone synthesis can become rate-limiting thereby interrupting the biochemical process that maintains media glucose homeostasis (Schaefer *et al*., 2001; Leheska *et al*., 2002). Pre-slaughter insufficiency of energy sources can then result in low lactic acid production and thus, high *post-mortem* pH_u (pH > 5.8) (Muchenje *et al*., 2008). At such pH, muscle proteins are above their iso-electric point (pH = 5.4) (Kerry *et al*., 2002; Muchenje *et al*., 2009b). Though above such point, there can be an increase in the muscle's ability to hold fluid molecules therefore minimising losses during processing (Morgan, 1995) however, such pork is aesthetically unpleasant due to the pronounced dark appearance at the cut surface (Perez *et*

al., 2002; Wulf *et al*., 2002). This can be due to the high altered absorption characteristic of myoglobin with poor light reflection thus decreasing acceptance of pork by the end users (Hambrecht *et al*., 2004; Ndou *et al*., 2011). Such a high pH_u can also be unfavourable for muscle conversion to pork and is liable for decreased microbial stability (shelf-life) (Cárdenas *et al*., 2008; Muchenje *et al*., 2008).

According to Morgan (1995) and Roldan-Santiago *et al*. (2013), though holding periods (lairage) less than an hour can cut DFD occurrences, previously incurred incidences (*viz*. human-animal interaction) of stress can increase the frequencies of light appearing pork cuts. Aziz (2004) and Kerner (2005) stated that resting pigs with high energy reserves for half an hour can be conducive for the occurrence of both blood-splash and PSE. This can be due to the circulating secretions (cortisols, CKs and catecholamines) remaining high in the body from the first few hours with human contact during transit (Grandin, 2000; Ndlovu *et al*., 2007; Muchenje *et al*., 2009b). According to Warriss *et al*. (1998), lairage times that are less than an hour or more than three hours do not reduce circulating stress indicators and therefore, reflect no benefits in terms of stress reduction. Secretion of stress related indicators (cortisols and CK) speed up the biochemical processes for energy. However, due to hypoxia (low O_2) post-slaughtering; muscle cells switch to anaerobic oxidation and thus, build-up lactic acid (Perez *et al*., 2002; Foury *et al*., 2005). High production of *post-mortem* lactic acid can decrease ultimate pork pH (Moberg and Mench, 2000; Cárdenas *et al*., 2008). Such a rapid formation of intra-muscular acidosis in combination with high *post-mortem* carcass temperature normally results in denaturation of structural proteins and a resultant drop in meat water retention, juiciness, as well as tenderness (Hambrecht *et al*., 2004; Jama *et al*., 2008; Lonergan, 2008; Hoffman and Fisher, 2010). This drop in meat tenderness can be due

to the formation of bonds between thin and thick filaments (actin and myosin, respectively) and thus occurrence of *rigor mortis* (inextensibility of muscle fibres) (Pietrzak *et al*., 2008).

2.5.5. **Stunning of pigs**

Prior to the slaughtering of pigs which is a first step in the transformation of muscle into edible pork (Muchenje and Ndou, 2011), pigs have to be stunned depending on the religion of slaughterhouse operators and their clients (Grandin and Regenstein, 1994). Pig stunning is any mechanically, electrically or gaseous means of making the pig unconscious such that there is enough loss of blood to cause death from lack of oxygen to the brain and with high insensitiveness to pain during sticking (Yiu *et al*., 2001; Kerner, 2005). Stunning mechanically can be achieved through the use of the captive bolt method. On the other hand, stunning with electricity can be achieved by passing a certain current (*viz*. 1.2 Amps; 220 V; 5 seconds) across the brain (Grandin and Regenstein, 1994; Yiu *et al*., 2001; Belk *et al*., 2002). Stunning by gas can be achieved by immersing pigs in concentrations of gases such as the principal agent-carbon dioxide (recommended 45-75sec; 65-70% CO_2) (Belk *et al*., 2002; Kerner, 2005).

A study by the Department of Agriculture (DOA) (2004) revealed that after captive bolt stunning, not only will unconsciousness occur, but also damage of intracranial blood vessels and forced visible pieces of brain and other tissues will be dislodged into the circulatory system. According to Anil *et al*. (2002), the damaged intracranial blood vessels, whilst the heart is still pumping for several minutes, can result in the spreading throughout the body of some central nervous system material which enters the jugular venous blood. This can affect *post-mortem* pork appearance. On the other hand, the pieces of brain tissues as a result of the head trauma could also block the blood vessels. Such blockage can be noticeable in the

jugular venous blood within 30 seconds after stunning (Anil *et al*., 2002) and this can cause low exsanguination during sticking thereby creating a preferred medium for the survival of microbes (Chambers *et al*., 2001). Kerry *et al*. (2002) and Channon *et al*. (2003) supported this by noting that besides the risk of vertebral fractures and increased worker's risks due to excessive movements; exsanguination can be less effective after mechanical stunning. However, due to the injured head, the creatine kinase brain isoenzyme (CK-BB), a brain-specific protein's level can become elevated (Garland *et al*., 1996). This protein hastens the conversion of creatine to phosphocreatine (Kannan *et al*., 2002). Phosphocreatine will then serve as an energy reservoir for the rapid regeneration of adenosine triphosphate (ATP) which stores energy in the live muscle (Grigor *et al*., 2004). However, due to hypoxia at exsanguination, the synthesis of mitochondrial ATP to counteract such physiological stress from injury can become inhibited and thus, falls below the 5mM required to keep muscle relaxation (Garland *et al*., 1996). At such low concentrations of ATP, the muscle filaments actin and myosin combine to form a reversible actinomyosin complex and the muscle enters into an irreversible contracting state called *rigor mortis* thus a less tender meat cut is produced (Garland *et al*., 1996). As explained by Hall and Bradshaw (1998) and de Perre *et al*. (2010), the greater the physical physiological stress; the higher the secretion of stress-related indicators into the fluid media, which in turn can hasten *post-mortem* muscle glycolysis to result in a rapid pH drop, and thus decreased tenderness.

On the other hand, gas (CO_2) stunning can improve the worker's safety due to the less kicking by the pigs during the shackling process (Kerry *et al*., 2002). However, during its inhalation carbonic acid (HCO_3^-) forms thereby lowering the muscle's pH_u which can increase the incidences of PSE (Hambrecht *et al*., 2004). Kerry *et al*. (2002) supported this by stating that even though the pH can be the same after 24-hours, CO_2 stunning leads to

metabolic acidosis and as a result, reduce initial muscle pH readings than electrically and mechanically stunned pigs.

2.6. **Correlations between Quality Parameters**

Many researchers (Hambrecht *et al*., 2004; Mach, 2008; Muchenje *et al*., 2008, 2009a; Sabuncuoglu *et al*., 2011; Chulayo and Muchenje, 2013; Gajana *et al*., 2013; Seshoka *et al*., 2013) have reported on the importance of glucocorticoids, CK and catecholamines as indicators of stress and how muscle pH_u (which affect meat quality traits) could be improved if influences of poor farm handling, transportation and slaughterhouse practices were to be avoided. Muscle ultimate pH is arguably, the most important pork quality parameter (Becker *et al*., 1989; Lonergan, 2008; Ndou *et al*., 2011). The aptitude of hydrogen ion (H^+) concentration in regulating pork proteins to hold fluids under the application of external forces can be seen as an example of a possible relationship with other pork quality traits. For instance, when the pork pH changes from the value 5.8, the charge of protein side-groups which may be positive or negative also changes (Heap *et al*., 1998; Muchenje *et al*., 2009b). Such changes then bring more charges for muscle proteins to hold fluid molecules around them thereby improving water-holding capacity (WHC) of the pork and so minimum liquid losses to occur during processing (Heap *et al*., 1998; Bertram *et al*., 2004; Hoffman *et al*., 2007). However, when the value of pH approaches 5.4 which is also referred to as the iso-electric point; the muscle proteins become least able to bind fluid molecules due to the limited space created intramuscularly. This space is minimised by the cohesion of actin and myosin (principal muscle proteins) filaments at that point and can cause a fluid loss as drip during storage and upon cooking. The resultant pork cut becomes tougher and drier when consumed by the end users due to the diminished ability to retain fluids and the low calpain activity to tenderise pork at low pH (Silva *et al*., 1999; Kerry *et al*., 2002; Lonergan, 2008;

Muchenje *et al*., 2009a). Varnam and Sutherland (1995), Bee *et al*. (2006) and Carlin *et al*. (2006) findings supported this and these authors also noted that in conjunction with elevated ionic strength and media metabolites, calpain–inhibitor (calpastatin) can partially gain more activity over calpain at a low muscle pH_u (< 5.6). Geesink and Koohmaraie (1999) and Lonergan *et al*. (2009) further elaborated that this loss of activity by Calpain results in reduced proteolysis and therefore, less tender meat.

On the other hand, Xiong *et al*. (1999) and Grandin (2000) established that when the muscle pH approaches a value of 6.0, pork tenderness decreases whilst the latter will start to increase as the pH increases from 6.2 to 6.6. Although at high muscle pH (pH >5.8) the Calpain enzymes gains more activity and tenderises meat, the meat becomes dark in acuity (Silva *et al*., 1999). This according to Hambrecht *et al*. (2004) and Ndou *et al*. (2011) is due to the accumulated surface moisture which reduces the amount of light reflected whereas there is an altered absorption characteristic by myoglobin in the pork cut.

2.7. **Summary of Review**

Pig transformation to the desired pork meat quality can be costly and risky to all stakeholders involved (farmers, truckers, processors and consumers), and can also be a strenuous exercise to the pig itself. This process should therefore be a joint responsibility between those stakeholders involved to improve their practices in from pig reproduction to pork production in order to maximise gains over the expenses (capital and health related expenses) and thus better satisfaction for all.

The amount and type of pre-slaughter stress experienced by the pig prior to slaughter can initiate the secretion of enzymes and hormones (CK and cortisol, respectively) or their metabolites which mobilises muscle energy sources. Pigs that respond actively to such

stressors can have energy levels that have deviated-from-normal levels and subsequently, deviated-from-normal *post-mortem* muscle pH_u. The deviation from normality also affects the dominant pork sensory features (colour, tender, flavour and juiciness) which contribute to a consumer's satisfaction. Thus, the industry and the nation experiences economic losses because of poor pig welfare and pork quality hence the following chapters will tabulate the findings of the current research on the effects of pre-slaughter stress on the levels of cortisol, creatine kinase and their subsequent relationship with pork quality.

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Chapter 3: Effect of Sex and Time to slaughter (transport and lairage duration) on the levels of Cortisol and Creatine Kinase in Crossbred Pigs

Abstract

The study was conducted to determine sex and time to slaughter (transport and lairage duration) effects on the levels of cortisol and creatine kinase in 60 (22 weeks old) commercial crossbred pigs. Saliva samples were collected between 07:00-10:00 the day before the 120km road journey, on arrival at the abattoir, and after a 20 hour lairage period. Cortisol levels from saliva, serum and urine (the last two taken on the slaughter line) were measured from all pigs using a cortisol enzyme immunoassay kit. Creatine kinase levels were determined from serum samples. Boars had high (P <0.05) saliva cortisol levels after transportation. In both sexes, saliva cortisol significantly increased (P <0.001) in response to time to slaughter (transport and lairage duration). There was a significant (P <0.05) interaction of sex and time to slaughter on serum cortisol. However, it was only the effect of time to slaughter which showed urine cortisol to be high (P <0.05) in pigs that were slaughtered on arrival. Likewise, it was only the effect of sex that showed high (P <0.05) serum creatine kinase levels in gilts. Correlation analysis showed no (P >0.05) relationship between farm saliva cortisol and cortisol levels from serum and urine. Saliva cortisol after transportation showed significant positive correlation with urine cortisol levels. It was therefore concluded that responses of the Hypothalamic-pituitary-adrenal axis shown by cortisol in saliva, serum and urine, and serum creatine kinase following time to slaughter (transport and lairage duration) between gilts and boars can be used to determine stress in pigs.

Keywords: Boars, gilts, saliva; serum; stress; urine

3.1. **Background**

Pork meat and its products are known to be more palatable than those made from beef and mutton and are valued for their nutritional properties such as polyunsaturated fatty acids (*viz*. linoleic and linolenic) (Lesser, 1993; Wood *et al*., 2008; Okrouhla *et al*., 2010). However, besides such admirable properties, pork can be enhanced by limiting pig exposure to stress during handling en route to the abattoir, and carefully assessing the effects of different *ante-mortem* actions (Gajana *et al*., 2013). Though responses to transit and lairage events are gene-dependent factors; they can be influenced by other environmental factors such as substandard transport and/ or lairage durations or mixing of animals (Grandin, 1994; Aziz, 2004; Correa *et al*., 2010).

In addition to environmental challenges, substandard transport and lairage durations (3>hours<3) tend to place greater demands on physiological systems (energy metabolism and fluid regulation) and thus, elevate hormones and creatine kinase secretion in pigs to improve fitness (Becker *et al*., 1989; Warriss *et al*., 1998; Schaefer *et al*., 2001; Richardson, 2002; Grandin, 2007; Mota-Rojas *et al*., 2009; Faucitano, 2010). Rapid elevation of stress-related indicators can thereafter cause vasoconstriction, especially in high temperature seasons (in excess of 15^{o}C), that can hinder heat dissipation (Romero and Butler, 2007). Findings by Southern *et al*. (2006) and Gonzalez *et al*. (2007) supported this and these authors also noted that in addition to exhausted metabolic muscle and dehydration, heat stress is a consequence of transport, especially with high stocking densities and poor ventilation. According to Pérez *et al*. (2002), the muscle of a stress-susceptible pig can be over-reactive to stressful handling. Such muscle can be prone to excessive catabolic activities which activate glycolysis and block glucose uptake by peripheral tissues. The pig can therefore develop hyperthermia and lethal blood metabolites (*viz*. potassium and hyperglycaemia) (Vasconcellos *et al*., 2011; Owens, 2012).

However, as in any other farm animal species, most of the concerns when assessing the pig's well-being are linked with human-animal interaction and surroundings (Schonreiter and Zanella, 2000; Deen, 2005). According to Bennett *et al*. (2008), Bodnariu (2008) and Escribano *et al*. (2012), the psychological or physiological stress encountered by pigs during their switch to pork can be evaluated on the status of the hypothalamic-pituitary-adrenal (HPA) axis. This was supported by Smith and French (1997) and Van de Perre *et al*. (2010) who stated that the activity of the HPA axis and sympathetic nervous system (SNS) can be increased due to human-animal interaction, mixing different sexes or animals from different origins and or exposure to unfriendly settings/environment. This may lead to a great increase of stress initiated secretions in the circulatory system which are known to have a glycogenolysis and gluconeogenesis effect (Hoffman and Laubscher, 2011). This can impair pork quality through anaerobic glycolysis and the production of hydrogen ions (measured as ultimate pH) and thus, decrease eating quality (Geverink *et al*., 1998; Choi *et al*., 2012).

Though there are many methods (*viz*. saliva, blood, urine, faeces and hair) to measure stress in pigs, saliva sampling is a non-invasive and a stress-free method compared to other methods which may pose various problems (*viz*. complicates results interpretations or may be unethical) due to lack of superficial blood vessels in the species (Bennett *et al*., 2008; Bodnariu, 2008; Hillmann *et al*., 2008). Other methods may be impractical (measuring heart rate) and or time consuming (behavioural parameters) (Escribano *et al*., 2012; Seshoka *et al*., 2013). The objective of this study was to determine the effect of sex, transportation and lairage duration on the levels of cortisol and creatine kinase in crossbred pigs.

3.2. **Materials and Methods**

3.2.1. **Ethical Consideration**

The study was done following normal routine farm to abattoir practices and conditions and all experimental procedures were according to the ethical principles of experimentation established by the Committee of Ethics on Animal Use of the Society for the Prevention of Cruelty to Animals (SPCA).

3.2.2. **Site Description**

The study was conducted at the University of Fort Hare Farm's Piggery Trust unit. The farm is located 32º.48` S (latitude) and 26º.53` E at 520 m above sea level (Eastern Cape Province, South Africa).

3.2.3. **Animal management**

Sixty 22 weeks old commercial crossbred pigs (30 Duroc boars x 30 Dutch Landrace-Large White gilts) were used in the study. All pigs were reared on the farm and given water from nipple drinkers and fed a commercial diet *ad libitum* until transported to the abattoir. They were divided randomly and raised in four partly-slatted pens (15 pigs per pen) of the same sex and housed in the same building. Standard temperature in the building was set at 19.5ºC, and artificial light was provided from 07:00 to 17:00. All batches were handled at the farm (Fort Hare Piggery Trust) under identical conditions.

3.2.4. **Transportation details**

Sixty crossbred pigs (30 boars vs. 30 gilts) were transported to East London abattoir under the same handling conditions. In brief, all the pigs were handled and loaded from Fort Hare Piggery Trust farm using an electric goad and were not off-loaded during journey breaks. The pigs were stocked at an average loading density of 0.8 m^2 per animal (Warriss, 1998). The

journey commenced at 10:00 am, and there were no separations by sex or loading by pen which thus meant that full mixing occurred. A truck equipped with natural ventilation and with slatted sides and a slatted floor to avoid slipping was used for the transportation of pigs. There was one driver transporting pigs to the abattoir. Pigs were transported on a tarred road for two hours to the East London abattoir which is close to 120 km away from the farm.

3.2.5. **Lairage details and Slaughter procedure**

Thirty pigs (15 boars vs. 15 gilts) were housed for 20 hours in roofed pens with no feed available, having only tap water *ad libitum*, the other 30 (15 boars vs. 15 gilts) were slaughtered on arrival (zero lairage). The pigs were randomly selected according to their sex and slaughtered in four batches (15 pigs per batch) in accordance with approved commercial procedures of the abattoir. The live slaughter weight for gilts was approximately 76.9 ± 5.7kg while boars weighed on average 78.3 ± 5.3 kg. An electric prodder was used to move the pigs from the pens to the slaughter area. The 15 pigs per batch were placed into a room, stunned with head-only electric stunner method (110 V and 8 A) for 3-5 seconds, shackled and then bled by sticking within 30 seconds. The carcasses were immersed in warm water with a regulated temperature of 60 ^{0}C. The hair from the carcasses was removed by mechanical tumbling, followed by evisceration and inspection of the carcasses by the authorized meat inspection personnel.

3.2.6. **Sampling Procedures and Chemical Analysis**

3.2.6.1. **Saliva sampling**

Three saliva samples per pig were taken as shown in Figure 3.1: one basal sample from all pigs a day before transportation (between 07:00 and 10:00), on arrival at the abattoir from the

30 pigs selected to be slaughtered on arrival, and a day after before stunning from the remaining 30 pigs. Saliva samples were taken using Salivette tubes (Sarstedt AG & Co, Germany), allowing individual pigs to chew cotton balls attached to pieces of string for 1-2 minutes until the balls were thoroughly moistened (Seshoka *et al*., 2013). The cotton balls were then placed in the tube, transported (for 1:30 min.) on ice (4^{o}C) from the abattoir to the University of Fort Hare laboratory. The samples were centrifuged at 20^{o}C for 10 min at 3550 x g and stored at -20^{o}C until analyzed for cortisol.

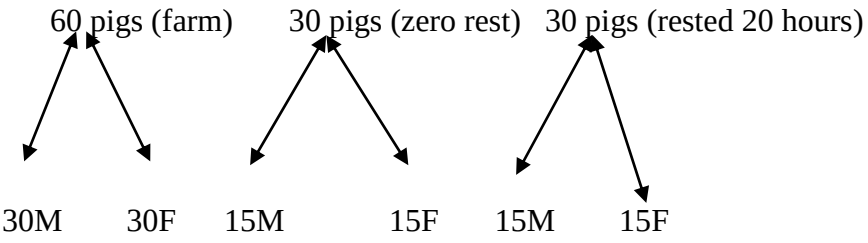

Figure 3.1: Method outlining fluid sample collection

3.2.6.2. **Blood collection, Serum separation, and Urine collection**

Serum samples were collected at the slaughter plant during exsanguination using 6.0 ml Vacutainer® tubes treated with anticoagulant. The samples were put on ice (4^{o}C) and transported to the laboratory. To remove debris and minimise the turbidity which can negatively impact on the accuracy of analysis (Mohamed *et al*., 2012; Salimetrics, 2012), the samples were centrifuged at 20^{o}C for 10 minutes at 3550 x g. Serum samples were transferred to Eppendorf tubes (1.5 ml) and stored at -20^{o}C until analysis for cortisol and creatine kinase. Urine samples were taken from the bladder of pigs on the slaughter line into hand-held plastic

cups, and labelled with the individual's number and sex. They were stored at -20^{o}C in separate vials until analysis for cortisol.

3.2.6.2.1. **Measurement of Cortisol levels in Saliva, Serum and Urine**

The levels of cortisol were determined using commercial cortisol enzyme immunoassay (EIA) kit for the in-vitro diagnostic quantitative determination of cortisol in plasma (Palme and Möstl, 1997; IBL International, 2013) according to manufacturer instructions. Saliva, and serum and urine cortisol concentrations were expressed in ng/ml, and nmol/L, respectively. The inter-assay coefficient of variation ranged from 16.06% to 16.34%, and the intra-assay coefficient of variation ranged from 9.5% to 11.0%.

3.2.6.2.2. **Measurement of Creatine Kinase level in Serum**

The levels of creatine kinase in stored serum were determined using a commercial colorimetric diagnostic kit (CK; IL Test kit, No. 181605-90), the Monarch 2000 Chemistry system (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium). Concentrations of creatine kinase in serum were expressed in units per litre (U/L). The inter-assay coefficient of variation was 5.15%, and the intra-assay coefficient of variation was 11.13%.

3.2.7. **Statistical analysis**

The effect of sex and time to slaughter (transport and lairage duration) and their interaction on cortisol and CK was analysed using the Generalised Linear Model Procedures of Statistical Analysis System (SAS, 2003). The model used was as follows:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$$

Where:

Y_{ij} is the observed response (dependent) variables

μ is the overall mean

α_i is the effect of time to slaughter (transport and lairage duration)

β_j is the effect of sex

$(\alpha\beta)_{ij}$ is the interaction effect of time and sex on cortisol and creatine kinase activity

ε_{ij} is the experimental error due to other factors

One-tailed, paired Student's *T* test was used to test for statistically significant differences in cortisol changes in the saliva in response to the treatments. Pearson's correlation coefficients among cortisol levels from the media were determined using SAS (2003). Significant differences among group means were tested by least significant differences and differences at $P < 0.05$ were considered to be statistically significant. Data were presented as the means ± standard error of the means. Cortisol from saliva was normalized by log-transformation.

3.3. **Results**

3.3.1. **Effect of Sex and Time to slaughter (transport and lairage duration) on the levels of Cortisol in Saliva.**

From the 22 weeks old pigs which were slaughtered after a 120km drive, there were no significant (P >0.05) differences in baseline saliva cortisol levels (ng/ml) between gilts and boars (3.9 ± 0.06 ng/ml vs. 4.5 ± 0.06 ng/ml, respectively) (Figure 3.2). There was a difference (P <0.001) between gilts and boars (15.2 ± 0.07 ng/ml vs. 38.6 ± 0.07 ng/ml, respectively) on the levels of cortisol during the time of slaughter (after transportation) (Figure 3.2). However, statistical analysis showed no significant (P >0.05) differences between gilts and boars (16.3 ± 0.06 ng/ml vs. 16.5 ± 0.06 ng/ml, respectively) in terms of saliva cortisol response after 20 hours of lairage time (Figure 3.2). A difference (P <0.001) between the levels of baseline saliva cortisol and cortisol levels at the time of slaughter (transport and lairage duration) as tested by Paired *T*-test analysis was observed.

3.3.2. **Interaction of Sex and Time to slaughter on the levels of Cortisol in Serum and Urine, and Creatine Kinase in Serum**

Regarding measurements from serum and urine, the interaction of sex by time to slaughter was significant (P <0.05) on the levels of cortisol in serum (Figure 3.3). Though there was no interaction (P >0.05) observed in levels of urine cortisol on pigs not rested, the interaction was significant on pigs rested for 20 hours (Figure 3.4). There was no interaction of sex and time to slaughter on the levels of serum creatine kinase (Figure 3.5).

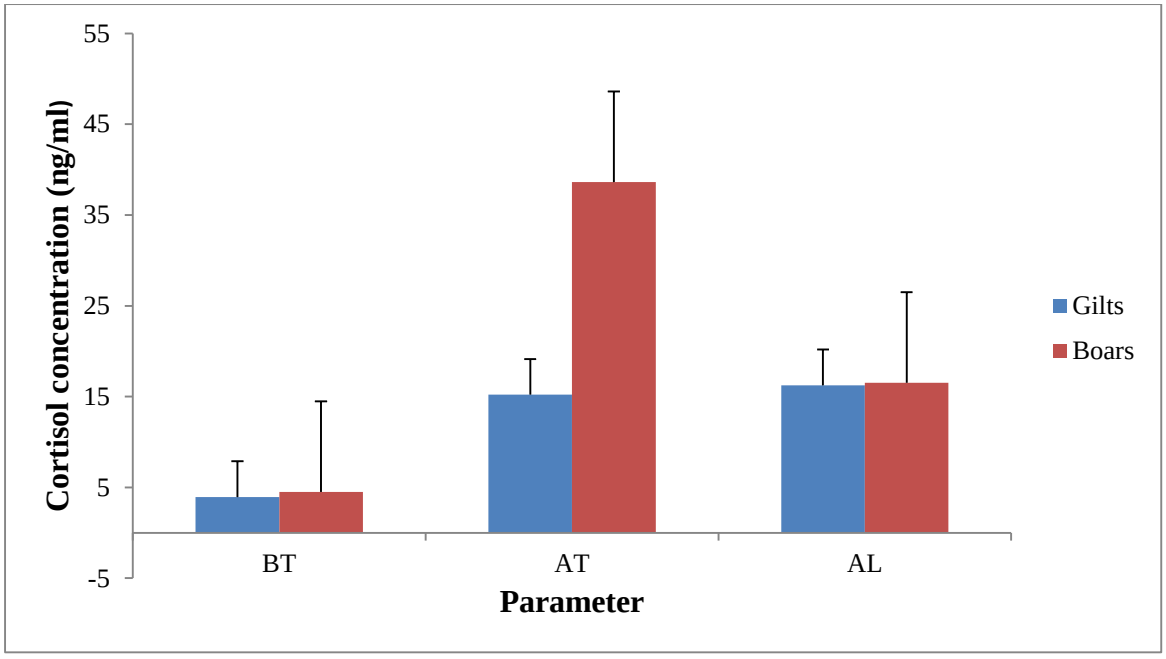

Figure 3. 2: Mean (standard error of the mean) cortisol levels in saliva (ng/ml) from pigs before transportation (BT) (N = 60), after transportation (AT) (N = 30) and after 20 hour lairage (AL) (N = 30), sampled in the home pen and at the abattoir (*P* <0.001).

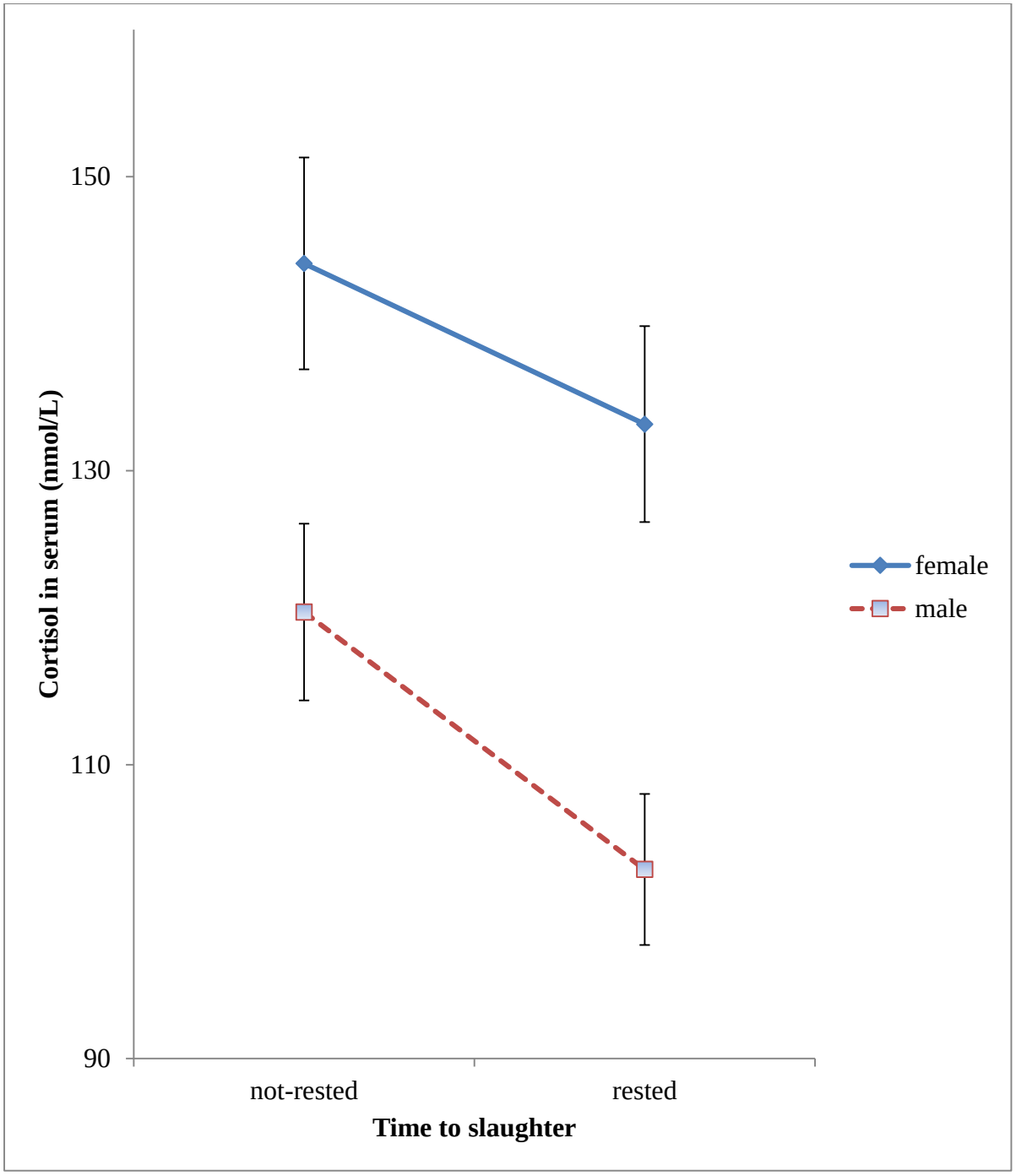

Figure 3. 3: Interaction of sex and time to slaughter on serum cortisol (nmol/L); not rested = slaughtered on arrival (zero lairage duration), rested = 20 hours of rest.

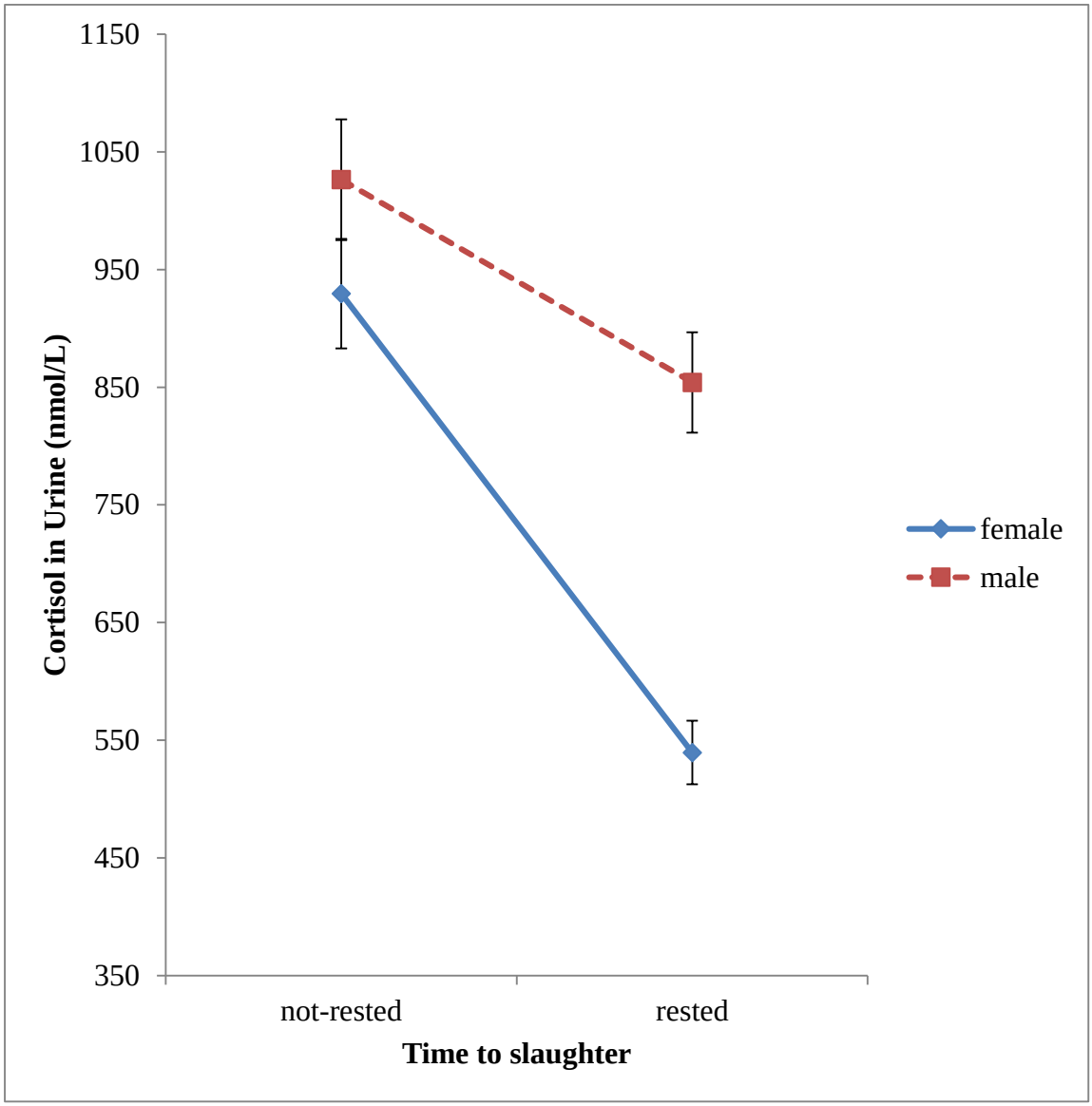

Figure 3. 4: Interaction of sex and time to slaughter on urine cortisol (nmol/L); not rested = slaughtered on arrival (zero lairage duration), rested = 20 hours of rest.

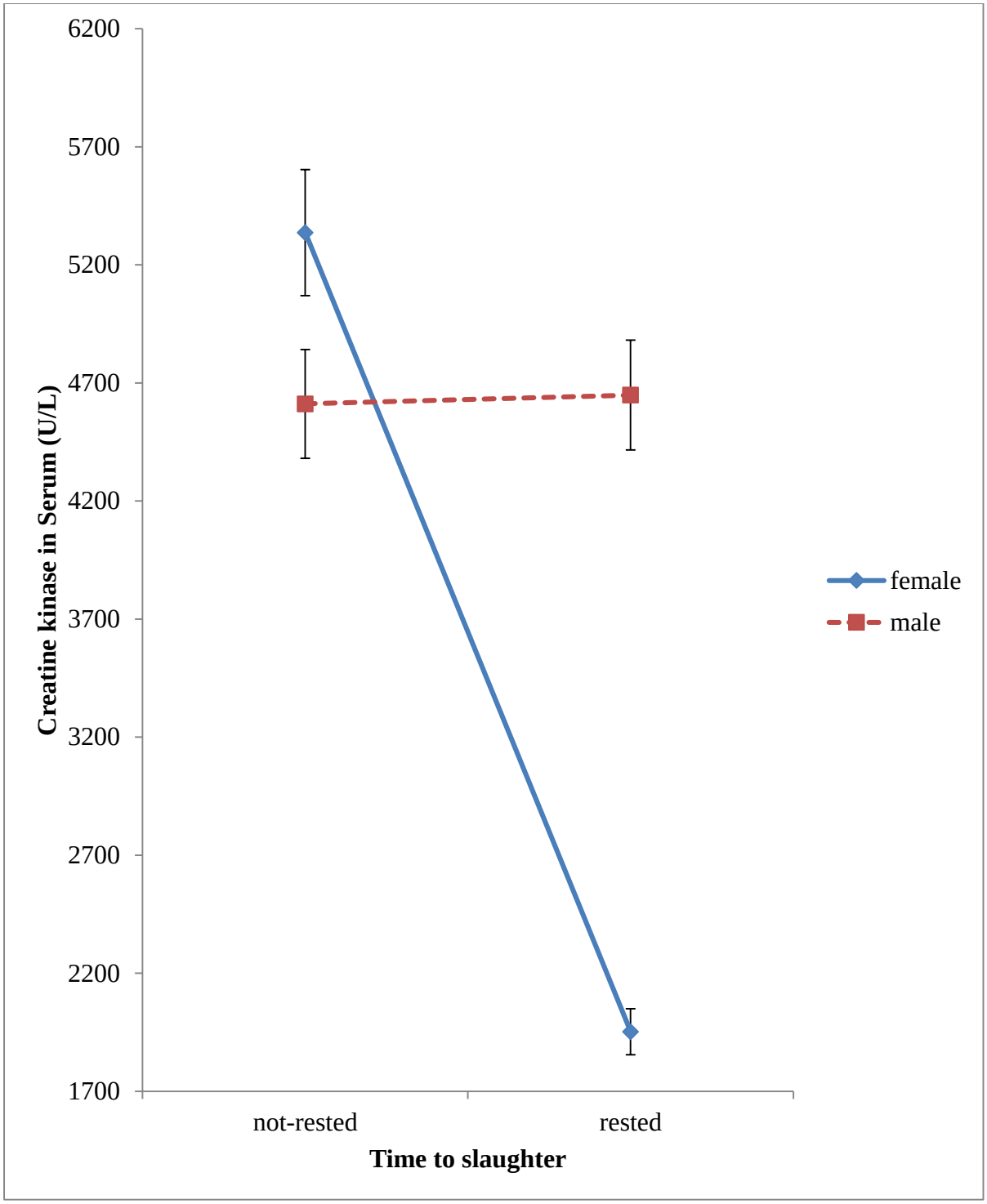

Figure 3. 5: Interaction of sex and time to slaughter on serum creatine kinase (U/L); not rested = slaughtered on arrival (zero lairage duration), rested = 20 hours of rest.

3.3.3. **Effect of Sex and Time to slaughter (transport and lairage duration) on the levels of Cortisol in Serum and Urine, and Creatine Kinase in Serum**

The effects of sex and time to slaughter were confirmed (P <0.05): on serum creatine kinase (U/L) for sex (138.6 ± 8.05 U/L gilts vs. 109.6 ± 8.18 U/L boars) (Table 3.1) and urine cortisol (nmol/L) (977.9 ± 96.14 nmol/L not rested vs. 699.8 ± 73.43 nmol/L rested) for time to slaughter (Table 3.2).

Table 3. 1: Levels of cortisol (nmol/L) in serum and urine, and creatine kinase (U/L) in serum (LSMeans ± standard error of the mean) between gilts and boars.

Physiological parameters	**Gilts (**n=30**)**	**Boars (**n=30**)**	***P*-**value
Serum cortisol	3644.33±505.601	4550.10±524.687	0.2188
Urine cortisol	734.50±107.489	940.25±55.507	0.1031
Serum creatine kinase	138.63±8.052	109.55±8.177	0.0141

Table 3. 2: Levels of cortisol (nmol/L) in serum and urine, and creatine kinase (U/L) in serum (LSMeans ± standard error of the mean) between not rested and rested pigs.

Physiological parameters	**Not rested (**n = 30**)**	**Rested (**n = 30**)**	***P*-**value
Serum cortisol	4895.03±505.601	3300.30±524.687	0.0330
Urine cortisol	977.95±96.141	696.80±73.429	0.0298
Serum creatine kinase	130.16±8.035	118.02±8.177	0.2941

3.3.4. **Pearson correlations between Cortisol levels in Saliva, Serum and Urine**

Pearson correlations were run to assess the relationships between cortisol levels in saliva, serum and urine from the 30 boars and 30 gilts aged 22 weeks (Table 3.3). Correlations were not significant (P >0.05) for the other parameters. However, saliva cortisol after transportation (r = 0.52) was positively related with urine cortisol after slaughter (P <0.05).

Table 3. 3: Pearson correlation coefficients (*r*) among cortisol levels from saliva, serum and urine.

Variables	BSCBT	SCAT	SCAL	Serum cortisol	Urine cortisol
BSCBT	-	-0.12	0.17	-0.004	-0.08
SCAT		-	-0.03	0.26	0.52*
SCAL			-	0.10	-0.09
Serum cortisol				-	0.09
Urine cortisol					-

* Indicate significance at $P < 0.05$. BSCBT = Baseline saliva cortisol before transportation; SCAT = Saliva cortisol after transportation; SCAL = Saliva cortisol after lairage duration

3.4. **Discussion**

From this study, the significant increase (P <0.001) in levels of saliva cortisol after transportation and lairage duration from all pigs agrees with reports by Schonreiter and Zanella (2000), Chaloupková *et al*. (2007), Smiecińska *et al*. (2011) and Escribano *et al*. (2012). According to Na-Lampang (2013), this can be due to the (un)loading procedures, transport and lairage durations and exposure to new settings which tend to have significant effects on stress-related indicators of the pig. According to Grigor *et al*. (2004), Zhen *et al*. (2012) and Miranda-de la Lama *et al*. (2014), human-animal interactions and/or all the activities associated with transport and lairage duration can induce physiological reactivity of the pig's nervous system and thus, compromise baseline levels of stress-related indicators.

The significant differences between sexes in terms of saliva cortisol response at time of slaughter (after transport) could suggest that boars were more reactive to transportation compared to gilts. This is in line with Ruis *et al*. (1997) and de Groot *et al*. (2001) who reported higher cortisol levels in castrated pigs than in gilts after strenuous exercise. According to Moberg and Mench (2000) and Faucitano and Schaefer (2008), high cortisol levels in males compared to females can be due to high reaction of boars in situations when threat to physical or mental homeostasis is perceived. Though there was a decrease in saliva cortisol levels after lairage duration; sensitivity to its stress did not differ between gilts and boars. This concurs with other studies and is supported by Sutherland *et al*. (2005) and GarcÃa-CeldrÃ¡n *et al*. (2012) who reported that although lairage time could help animals to recover from transport associated stress, its effect between sexes is insignificant.

The significant interaction of sex by time to slaughter on serum cortisol and urine cortisol after 20 hours of rest is in agreement with Smith and French (1997) who also reported significant interaction of sex by stress in cortisol values in urine between male and female

monkeys. They further stated that higher cortisol values due to a nerve-racking event, especially in female animals can be due to their high circulating levels of ovarian steroids (*viz*. estrogens). The absence of significant sex by time to slaughter interaction for serum creatine kinase may be suggesting that time to slaughter had little or no effect in muscular recovery. This was discussed by Cook *et al*. (1998), Partida *et al*. (2006), Faucitano and Schaefer (2008), Smiecińska *et al*. (2011) and Peres *et al*. (2014) who noted that pre-slaughter rest period should correspond to transport duration, otherwise, lairage intensity can entail high levels of stress-related indicators (*viz*. lactate, creatine kinase and cortisol) at slaughter in the blood. Our study findings are however, in contrast with Averos *et al*. (2007) who found a significant recovery of creatine kinase in the lairage, particularly by males. In this study, it was only the effect of sex in serum creatine kinase which remained significant, pointing to gilt's physical contact with boars which the latter can have an aggressive temperament. This theory is supported by Pearce and Hughes (1987) and Perez *et al*. (2002) who reported that for stress to be elicited in the gilt, a full physical contact between the boar and the gilt is a necessary prerequisite. Such contact may result in abrasions, fatigue and fear and thus, elevate the secretions of creatine kinase and cortisol in the gilts (Delezie *et al*., 2007; Ndlovu *et al*., 2007). As observed in the stunning race, many gilts had a propensity of urinating more often and as a result, many of them did not have urine in their bladder after evisceration. Therefore, differences in sex behavioural patterns are particularly interesting and warrant further investigation.

The observed non-significant correlation between baseline saliva cortisol, saliva cortisol at slaughter and cortisol levels from serum and urine is supported by Beerda *et al*. (1996) who found no significant relationship between urinary cortisol and plasma cortisol under undisturbed conditions. Conversely, the positive correlation between saliva cortisol at

slaughter (after transportation) and cortisol in serum (though not significant) and urine cortisol (significant) after slaughter was expected since pre-slaughter handling can induce physiological reactivity of the nervous system. This observation was supported by Beerda *et al*. (1996) and Grandin (1997) who noted that pre-slaughter handling in living organisms can increase both heart rate and motor activity at time of slaughter and that can influence the subsequent properties of pork.

3.5. **Conclusions**

Saliva cortisol results imply that boars are more reactive to pre-slaughter transportation stress compared to gilts. However, more studies should be designed to investigate the effect of transportation by sex or pens as physical contact between sexes might influence the results. Comparison of the combined factors of sex by time to slaughter confirmed our previous knowledge of the individual effects of these two factors. In this study, it was noticed that lairage duration has a significant effect on serum and urine cortisol levels. Hence, it can be concluded that lairage duration alleviated psychological stress and associated handling in pigs. The high levels of serum creatine kinase in gilts, suggest that they were more reactive to physical contact compared to boars. The lack of significant correlation between baseline saliva cortisol and cortisol levels in saliva at the time of slaughter and in serum and urine showed that pigs were not subjected to any stress during saliva collection. However, saliva cortisol (after transportation) showed significant positive correlation with urine cortisol levels, which could suggest that the more pigs are transported, the greater the stress experienced, as indicated by high cortisol levels after transportation. It can therefore be concluded that sex and time to slaughter (transport and lairage duration) have an effect on the levels of cortisol and creatine kinase in crossbred pigs.

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Chapter 4: Effect of Sex and Lairage duration on Pork quality

Abstract

This study determined the effect of sex and lairage duration on the quality of pork in 60 (22 weeks old) commercial crossbred pigs. While some 30 pigs (15 boars vs. 15 gilts) were housed for 20 hours prior to slaughter after a 120 km road journey, the other 30 (15 boars vs. 15 gilts) were slaughtered on arrival after travelling the same distance. The effect of sex and lairage duration on meat pH, colour, thawing and cooking losses and tenderness was determined from the *Longissimus dorsi* muscle. Although sex by lairage duration interaction in muscle pH and colour values was of little significance, sex's effect on the L* value was significant. Effect of sex by lairage duration interaction was significant on meat cooking loss. The effect of lairage duration had no significant influence on any measured meat quality traits. Baseline saliva cortisol (r = -0.40) was negatively correlated (P <0.05) with the pork L* value. Saliva cortisol after lairage (r = -0.38 and r = 0.38) was correlated (P <0.05) with muscle pH_{45} and pork a* value, respectively. Serum cortisol (r = -0.35) was negatively correlated (P <0.05) with muscle pH_{45}. Correlation analysis also demonstrated significant (P <0.05) negative correlations between saliva cortisol after transportation (r = -0.35), saliva cortisol after lairage (r = -0.44), serum cortisol (r = -0.40) and meat cooking loss. The negative correlation may indicate that as the level of cortisol increases water-holding capacity decreases. It was concluded that the influence of transportation and lairage duration between sexes in terms of response to saliva cortisol and serum cortisol has an effect on muscle pH_{45}, colour lightness and cooking loss.

Keywords: Boars; gilts; colour; cooking loss; tenderness; muscle ultimate pH

4.1. **Background**

Prolonged stressors to pigs produce a host of physiological processes which in turn can interfere with normal behaviour and thus, be perceived as a danger to their welfare (Bennett *et al*., 2008; Chapter 3). Grandin (1994) and Aziz (2004) echoed similar sentiments by stating that including sex, exogenous factors to the animal (transport and lairage), though the response to these are gene-dependent, are the main causative agents for the development of aberrant pork quality. According to Van de Perre *et al*. (2010), pork defects are commonly caused by physical stress prior to stunning. Stress encountered by a pig during its conversion to pork tends to place demands for oxygen and increase the activity of the hypothalamic-pituitary-adrenal axis and sympathetic nervous system (Smith and French, 1997; Van de Perre *et al*., 2010). Such activities due to human-interaction or exposure to unsociable settings can elevate secretion of stress related indicators in the pig's circulatory system, which may in turn influence pork quality (Geverink *et al*., 1998).

It is widely recognised that carcass and physicochemical characteristics of pig meat, and thus pork quality can also be influenced by endogenous factors (*viz*. breed and sex) or their interaction with the environment (De Smet *et al*., 1996; Latorre *et al*., 2003; Hoffman *et al*., 2007; Daza *et al*., 2012; Mpakama *et al*., 2014). Hoffman *et al*. (2005), Zhang *et al*. (2010) and Franco and Lorenzo (2012) noted that due to many individual differences (*viz*. stress reactivity or fat and muscle deposition) between the same breed, sex has a greater effect on pork characteristics. For instance, male pigs tend to accumulate more fat and taint steroids than females (Squires *et al*., 1996; Wagener *et al*., 2006). This can cause variation between sexes as fat is very key to pork sensory traits (*viz*. juiciness and microbial stability) (Hoffman *et al*., 2005).

Although all livestock homeostasis is likely to be disturbed prior to slaughter physico-chemical characteristics (*viz*. water retention, colour and tenderness) have been reported to be poor in males compared to females (D'Eath *et al*., 2010; Choi *et al*., 2012). In pigs, this has been attributed to boars being more reactive to stress than gilts (Zhang *et al*., 2010). Vulnerability to stress may provoke metabolites secretion which can hasten *post-mortem* degradation of energy sources with consequent rapid glycogen depletion, and rapid changes in *post-mortem* muscle pH values (Jaturasitha *et al*., 2006; Choi *et al*., 2012). The post slaughter carcass body temperature is typically around 37-39^{o}C (Ercolini *et al*., 2009; Xu *et al*., 2011). Such temperatures in conjunction with reduced pH values (< 5.6) can affect muscle functionalities (*viz*. natural ability to retain fluids and reflect light) (Ercolini *et al*., 2009; Franco *et al*., 2011). Jaturasitha *et al*. (2006) noted that poor water retention in conjunction with pale colour and a lower score for marbling tend to be common incidences in male carcass grading compared to gilts. Pork sales are influenced by weight, price, previous consumption experience and good appeal to consumers. These parameters can directly translate into economic profit for the processors (Thu, 2006; Hoffman *et al*., 2009; Žemva *et al*., 2010). The objective of this study was, therefore, to determine the effect of sex and lairage duration on pork quality.

4.2. **Materials and Methods**

4.2.1. **Study site and study animals**

The study site and management of experimental pigs were as described in Section 3.2.

4.2.2. **Sampling Procedures and Chemical Analysis**

4.2.2.1. **Pork samples and Meat quality tests**

For pork quality analyses, a 2 cm-thick *Longissimus dorsi* (LD) muscle of the left side of the carcass was sectioned between the 10^{th} and 11^{th} ribs of the loin region after slaughter while the carcass was still hanging. After the initial pH measurement was taken (pH_{45}), the samples were vacuum packed and transported to the laboratory in a cooled, insulated box after which the following additional measurements were taken:

4.2.2.2. **Determination of muscle pH**

A portable pH meter (Crison pH 25, Crison instruments, S. A., Alella, Spain) equipped with an insertion glass combination electrode (Mettler Toledo Greifensee, Switzerland) was used to measure muscle pH at 45 minutes (pH_{45}) and 24 hours *post-mortem* (ultimate; pH_u). The pH meter was calibrated with pH 4 and pH 7 standard solutions. Values of muscle pH were conducted, applying the regimes previously described by Gajana *et al*. (2013).

4.2.2.3. **Determination of Pork colour (L*, a* and b*)**

Light reflectance scores for CIE (Commission Internationale de l'Eclairage) L* (lightness), a* (redness), and b*(yellowness) were measured at three locations of the LD using a Minolta colour-guide (model 45/0 BYK-Gardener GmbH machine, with a 20 mm diameter

measurement area and illuminant D65 and viewing angle 10°). Also, chroma (C*) value and hue-angle ($H_{b/a}$) were calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$, and $H_{b/a} = \tan^{-1}(b^*/a^*)$, respectively. Three readings were taken by rotating the Colour Guide 90° between each measurement, in order to obtain a representative average value of the colour. The guide was calibrated using the green standard (Lee *et al*., 2001; Gajana *et al*., 2013).

4.2.2.4. **Cooking Loss Components (CLC)**

Method: Immediately after slaughter before freezing, the *Longissimus dorsi* (LD) muscle samples (2 cm thick) were weighed and stored at -18°C. The samples were thawed over a period of 24 hours at 0 – 4°C. Thawed samples were blotted dry and weighed again. The same LD muscles were then placed into thin-walled plastic bags and placed in a water bath (85°C) until an internal temperature of 71°C (recorded by a portable thermometer) was reached. After cooking (1h: 25min), the LD muscles were taken from the plastic bags and cooled down at room temperature for 15 min before being blotted dry and weighed again for the cooking loss determination. Percentage thawing loss and cooking loss were calculated as follows:

Thawing loss % = [(weight before thaw – weight after thaw) ÷ weight before thaw] x 100.

Cooking loss% = [(weight of raw steak after thawing – weight of cooked steak) ÷ weight of raw steak after thawing] x 100.

4.2.2.5. **Pork tenderness**

Following cooking and one hour cooling, three sub-samples of one-cm-diameter were cored parallel to the grain of the meat. The samples were sheared perpendicular to the fibre direction using a Warner-Bratzler Shear Force (WBSF) device mounted on an Instron 3344 Universal Testing apparatus. Cross head speed was at 200 mm/min, one shear in the centre of

each core. The mean maximum load (N/1-cm) was recorded for each LD and used for statistical analysis.

4.2.3. **Statistical analysis**

The effect of sex, lairage duration and their interaction on pH; colour; thawing loss, cooking loss and tenderness was analysed using the Generalised Linear Model Procedures of Statistical Analysis System (SAS, 2003), using the following model:

$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$ where:

Y_{ij} = observed response (dependent variables) (pH, L*, a*, and b*, and CLC),

μ = overall mean

α_i = effect of lairage duration on the experimental units

β_j = effect of sex

$(\alpha\beta)_{ij}$ = the interaction of sex and lairage

ε_{ij} = random residual error distributed as ~N (0; Iδe^2).

Pearson's correlation coefficients among cortisol levels from saliva, serum and urine (data from Chapter 3), and meat quality parameters were determined using SAS (2003). Significant differences among group means were tested by Least Significant Differences (LSD) and differences at $P < 0.05$ were considered to be statistically significant. Data were presented as the means ± standard error of the means.

4.3. **Results**

4.3.1. **Effect of Sex by Lairage duration Interaction on pH, Colour, Thawing loss, Cooking loss and WBSF of Pork**

Interactions of sex and lairage duration on pH, colour, thawing loss, cooking loss and Warner-Bratzler Shear Force values of the sampled *Longissimus dorsi* (LD) muscles are shown in Table 4.1. The effect of sex by lairage duration interaction was significant (P <0.05) only on cooking loss of the LD muscle.

Table 4. 1: Least square means and standard errors of the means of sex and lairage duration interaction effect on measured variables of pork *Longissimus dorsi* muscle.

Factor	Sex	pH_{45}	pH_u	CIE L*	CIE a*	CIE b*	C*	($H_{b/a}$)	Tlos (%)	Cklos (%)	WBSF
Rested for	Gilts	6.27^{a}	6.00^{a}	27.77^{a}	19.93^{a}	10.70^{a}	22.66^{a}	28.09^{a}	11.20^{a}	27.71^{a}	21.59^{a}
20 hours	Boars	6.40^{a}	6.00^{a}	31.68^{b}	17.85^{a}	10.71^{a}	20.88^{a}	30.97^{a}	10.82^{a}	22.05^{ab}	18.96^{ab}
	SEM	0.084	0.096	0.946	0.785	0.592	0.867	1.244	2.023	2.606	1.3698
Slaughtered	Gilts	6.42^{a}	5.99^{a}	31.88^{b}	17.95^{a}	10.13^{a}	20.69^{a}	29.86^{a}	22.84^{b}	24.67^{ab}	15.37^{b}
on arrival	Boars	5.81^{b}	5.87^{a}	31.77^{b}	19.63^{a}	10.00^{a}	22.05^{a}	26.87^{a}	9.91^{ac}	18.48^{b}	19.52^{ab}
	SEM	0.113	0.130	1.281	1.063	0.801	1.173	1.685	2.739	3.528	1.855

^{ac}Means within a column without a common superscript differ ($P < 0.05$). pH_{45} = pH at 45 minutes of slaughter; pH_u = pH at 24 hours of slaughter.

CIE L* = Lightness; CIE a* = Redness; CIE b* = Yellowness; C* = Saturation index; $H_{b/a}$ = Hue-angle;

SEM = Standard error of the mean; Tlos = Thawing loss; Cklos = Cooking loss; WBSF (N) = Warner-Bratzler Shear Force

4.3.2. **Effect of Sex and Lairage duration on pH, Colour, Thawing loss, Cooking loss and Warner-Bratzler Shear Force of Pork**

The effect of sex on pH, colour, thawing and cooking losses and WBSF in the LD muscle is presented in Table 4.2. Though gilts appeared to have higher muscle pH_{45} and pH_u values than boars, statistically they did not differ (P >0.05). Boars (31.72 ± 0.82) showed higher (P <0.05) lightness (L*) values compared to the gilts (29.22 ± 0.82). Saturation index (C*) and Hue angle ($H_{b/a}$) values between the two sexes did not differ (P = 0.05). Sex effect on thawing and cooking losses was not significant (P >0.05). The Warner-Bratzler shear force, expressed in Newton units, did not differ (P =0.88) between sexes.

The notable differences between and among lairaged groups in values of muscle pH_{45} and pH_u: rested (6.34 ± 0.07 and 6.00 ± 0.07, respectively) and slaughter on arrival (6.11 ± 0.10 and 5.93 ± 0.09, respectively) was of little practical significance (P >0.05) (Table 4.3). Overall, there was no significant (P >0.05) effect of lairage duration seen in this experiment on colour, thawing loss, cooking loss and Warner-Bratzler shear force of the LD muscle.

Table 4. 2: Effect of sex on pH, colour, cooking loss components and Warner-Bratzler shear force in *Longissimus dorsi* muscle samples from boars and gilts (LSMeans ± standard error of the mean).

Muscle quality traits	**Gilts** (n= 30)	**Boars** (n= 30)	**SEM**
pH_{45}	6.32	6.19	0.08
pH_u	5.99	5.96	0.08
Lightness (L*)	29.22[a]	31.72[b]	0.82
Redness (a*)	19.23	18.48	0.65
Yellowness (b*)	10.50	10.46	0.47
Saturation index (C*)	21.97	21.29	0.70
Hue-angle ($H_{b/a}$)	28.71	29.52	1.04
Tlos (%)	15.31	10.50	1.86
Cklos (%)	26.64	20.79	2.07
WBSF (N)	19.40	19.16	1.19

[ab]Means within a row without a common superscript differ ($P < 0.05$). pH_{45} = pH at 45 minutes of slaughter; pH_u = pH at 24 hours of slaughter; Tlos = Thawing loss; Cklos = Cooking loss; WBSF (N) = Warner-Bratzler Shear Force. [a]SEM = Standard error of the mean

Table 4. 3: Least square means and standard errors of means of pH, colour, thawing loss, cooking loss and Warner-Bratzler shear force after Lairage duration.

Muscle Quality Trait	**SOA (**n = 30**)**	**Rested for 20 hours (**n= 30**)**	***P* –** value
pH_{45}	6.11±0.10[a]	6.34±0.07[a]	0.0685
pH_u	5.93±0.09[a]	6.00±0.07[a]	0.5286
Lightness (L*)	31.82±100[a]	29.73±0.74[a]	0.0993
Redness (a*)	18.79±0.78[a]	18.89±0.58[a]	0.9174
Yellowness (b*)	10.07±0.55[a]	10.70±0.41[a]	0.3597
Saturation index (C*)	21.37±0.84[a]	21.77±0.62[a]	0.7042
Hue-angle ($H_{b/a}$)	28.36±1.23[a]	29.53±0.91[a]	0.4539
Tlos (%)	16.37±2.20[a]	11.01±1.62[a]	0.0583
Cklos (%)	21.79±2.58[a]	24.88±1.91[a]	0.3428
WBSF (N)	17.45±1.36[a]	20.28±1.00[a]	0.1038

[a]Means within a row with common superscript do not differ ($P > 0.05$). SOA = Slaughtered on arrival; pH_{45} = pH at 45 minutes of slaughter; pH_u = pH at 24 hours of slaughter; Tlos = Thawing loss; Cklos = Cooking loss; WBSF (N) = Warner-Bratzler Shear Force

4.3.3. **Pearson's correlation coefficients between cortisol levels in saliva, serum and urine and meat quality traits**

In Table 4.4, Pearson correlations were run to assess the relationship between cortisol levels in saliva, serum and urine and meat pH, colour, thawing loss, cooking loss and tenderness in the 30 boars and 30 gilts aged 22 weeks. In terms of muscle pH, levels of cortisol in saliva after lairage (r = -0.38) and in serum (r = -0.35) were weakly negatively correlated (P <0.05) with muscle pH_{45}. There was a lack of significant (P >0.05) correlation between cortisol levels and measured muscle pH at 24 hours. With regard to meat colour, baseline saliva cortisol (r = -0.40) was negatively correlated (P <0.05) with the L* component of pork. Pearson's correlation coefficient indicated that saliva cortisol after lairage was positively correlated with colour redness (a*) (r = 0.38, P <0.05). Baseline saliva cortisol (r <0.5) showed no significant (P >0.05) correlation with thawing and cooking losses and Warner-Bratzler Shear Force. Correlation analysis also demonstrated significant (P <0.05) negative correlations between saliva cortisol after transportation (r = -0.35), saliva cortisol after lairage (r = -0.44), serum cortisol (r = -0.40) and meat cooking loss.

Table 4. 4: Pearson's correlation coefficients (*r*) among cortisol levels from saliva, serum and urine and measured pork quality variables

Variables	BCSBT	CSAT	CSAL	sCORT	uCORT	pH_{45}	pH_u	L*	a*	b*	C*	$H_{b/a}$	Tlos	Cklos	WBSF
BCSBT	-	-0.12	0.17	-0.004	-0.08	-0.07	-0.02	-0.40*	0.318	0.06	0.27	-0.17	-0.17	0.05	0.10
CSAT		-	-0.03	0.26	0.52*	-0.18	0.13	0.25	-0.24	0.04	-0.19	0.22	-0.12	-0.35*	0.21
CSAL			-	0.10	-0.09	-0.38*	-0.05	-0.24	0.38*	-0.01	0.31	-0.34	-0.10	-0.44*	-0.31
sCORT				-	0.09	-0.35*	-0.12	0.08	0.09	-0.21	0.01	-0.29	-0.16	-0.40*	0.12
uCORT					-	-0.11	0.26	0.19	-0.31	0.07	-0.23	0.35	-0.20	-0.23	-0.30
pH_{45}						-	0.21	0.16	-0.22	0.07	-0.15	0.25	0.24	0.25	0.12
pH_u							-	0.28	-0.20	0.13	-0.12	0.30	-0.07	0.03	0.28
L*								-	-0.46**	0.15	-0.32	0.55***	0.15	-0.24	0.03
a*									-	0.45**	0.96***	-0.38*	-0.24	-0.15	0.40
b*										-	0.69***	0.64***	-0.05	-0.11	0.10
C*											-	0.10	-0.21	-0.15	0.06
$H_{b/a}$												-	0.19	0.01	0.05
Tlos													-	0.27	-0.27
Cklos														-	0.15
WBSF															-

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; BCSBT = Baseline saliva cortisol before transportation; CSAT = Cortisol saliva after transportation; CSAL = Cortisol saliva after lairage; pH_{45} = pH at 45 minutes of slaughter; pH_u = pH at 24 hours of slaughter; sCORT = Serum cortisol; uCORT = Urine cortisol; sCK = Serum creatine kinase; L* = Lightness; a* = Redness; b* = Yellowness; C* = Saturation index; $H_{b/a}$ = Hue-angle; Tlos = Thawing loss; Cklos = Cooking loss; WBSF (N) = Warner-Bratzler Shear Force

4.4. **Discussion**

In this study, the absence of significant interaction between sex and lairage duration on muscle pH is in agreement with work by Hoffman and Laubscher (2011), Bureš and Bartoň (2012) and GarcÃa-CeldrÃ¡n *et al*. (2012) who found no significance for pH measurements among female and male animals. In terms of muscle pH_u values, they were within the acceptable range ($5.6 \geq pH_u \leq 6.\ 00$) according to Warner *et al*. (1997) and Mach *et al*. (2008). According to Heap *et al*. (1998), Bertram *et al*. (2004), Hoffman *et al*. (2007), Lonergan (2008) and Peres *et al*. (2014), meat acidity/pH can have a profound effect on meat quality by directly affecting the functions of proteins (*viz*. solubility, binding and reflection).

The absence of significance on colour measurements in this study is in agreement with work by other researchers (Latorre *et al*., 2004; Jaturasitha *et al*., 2006; Beattie *et al*., 2007) who found significant differences for colour values among groups to be of little practical significance. The capacity of muscle proteins to hold fluids after external forces have been applied can influence the meat's nutritional quality and its probability of being purchased (Jama *et al*., 2008). In this study, thawing loss and cooking loss were measured to determine the capacity of the sampled muscles to retain water after cooking. Despite them being not significant, the higher values for cooking loss in gilts than in boars were also reported by Latorre *et al*. (2004), Jaturasitha *et al*. (2006) and Đurkin *et al*. (2012) for *Longissimus dorsi* muscle. The absence of significant effect by lairage duration on cooking loss is in agreement with Toohey and Hopkins (2006) who found effect of lairage time on meat cooking loss to be insignificant. Lonergan (2008), Mach *et al*. (2008) and Đurkin *et al*. (2012) also reported that meat quality parameters including cooking losses and water-holding capacity can be predicted by pH_u, whilst higher levels of intramuscular fat as associated with males are negatively correlated with cooking loss.

Warner-Bratzler shear force (WBSF), a good predictor of tenderness in cooked meat (Liste *et al*., 2009) was used to determine tenderness of the sampled *Longissimus dorsi* muscles after cooking. In this study, neither sex nor lairage or their interaction was significant (P >0.05) on WBSF. These findings were in agreement with the reports by Liste *et al*. (2009) and Peres *et al*. (2014) who found no significance between boars and gilts, and no effect of lairage on tenderness. They attributed this to the insignificant variation both in muscle pH_u and liquid loss.

The demonstrated significant negative correlation between treatment saliva cortisol, serum cortisol and cooking loss may be explained by the status of the ionic content of muscle to hold natural water. According to Hedrick (1965), Kerry and Ledward (1999) and Tang *et al*. (2013), stress exposure prior to slaughter can alter muscle metabolism and subsequently, change the ultimate pH of the muscle (*viz*. 5.6 to 6.0) which can cause relatively great changes in meat colour and water-holding capacity. Hoffman and Laubscher (2011) echoed similar sentiments stating that high secretion of stress-related cortisol can alter muscle pH through its glycogenolysis and gluconeogenesis effects, coupled with protein degradation. The negative correlation between saliva cortisol after lairage, serum cortisol and muscle pH_{45} in our study may indicate that as cortisol levels increases due to stress, energy sources prior to slaughter would decrease, consequently high muscle pH_{45}. This is in line with findings by Okeudo and Moss (2005) which reported serum cortisol levels to be negatively related with pH_{45}. However, this is contrary to a study by Škrlep *et al*. (2009) which reported positive correlation between plasma cortisol and pH_{45}, which could be reflecting cortisol response to stress intensity prior to slaughter. Although there was no significant correlation between saliva cortisol levels and muscle pH_u, it is interesting that boars had a greater cortisol response to transportation which may indicate that, the higher L* value from boars is a result of greater cortisol response. Generally, the low lightness value (L* <40) observed between

sexes in this study may indicate that there was enough supply of muscle energy sources prior to slaughter hence, less pork lightness. This is supported by Škrlep *et al*. (2009) who noted that a positive correlation of cortisol levels with pH_u indicate low levels of glycogen in the muscle prior to slaughter hence, higher pH_u (pH_u >5.8). The observed negative significant correlation between pork lightness (L*) and redness (a*) can be due to the high muscle ultimate pH (pH_u >5.8) and status of the muscle protein. This is in agreement with Kannan *et al*. (2003), Hoffman and Laubscher (2011) and Kadim *et al*. (2013) who reported that a low lightness (L* <40) colour component and high redness (a*) can be due to the increase in meat pH_u and myoglobin content and thus, darker meat.

4.5. **Conclusions**

In the present study, sex had an effect on the meat lightness component (L*). Our study demonstrates significant sex by lairage duration interaction on meat cooking loss. In this study, lairage duration had no influence on any measured meat quality traits. Correlation analysis revealed a negative correlation between muscle pH_{45} and saliva cortisol after lairage duration and serum cortisol. With regard to meat colour, a negative correlation between the L* component of pork and baseline saliva cortisol was observed. In contrast, a positive correlation was observed between meat redness (a*) component and saliva cortisol after lairage. The negative relations between saliva cortisol after transportation and lairage duration, serum cortisol and meat cooking loss could suggest that as cortisol secretion increases due to stress, the ability to hold fluids after external forces decreases. It was therefore concluded that transportation and lairage duration between sexes, saliva cortisol and serum cortisol had an effect on muscle pH_{45}, colour lightness and cooking loss.

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Chapter 5: General Discussion, Conclusions and Recommendations

5.1. **General Discussion**

Commercial animals will always encounter potential stressors (*viz*. transportation, lairage time, stunning and many other factors) before slaughter that can impair welfare and reduce production efficiency even if the conditions are standardized (Delezie *et al*., 2007; Liste *et al*., 2009; Adzitey, 2011; Chulayo *et al*., 2012). Activation of the animals' body sympathetic nervous system (SNS) and Hypothalamic-Pituitary-Adrenal (HPA) axis by changes or stimuli from the environment can disturb the baseline levels of stress-related indicators (cortisol and creatine kinase). This is because of the physiological and psychological response an animal has to show to counter-act for survival, and in consequence, that has a negative impact on pH and quality of meat (Romero and Butler, 2007; Brannon *et al*., 2014). In this study, the methods of interests to measure response to the stimuli were cortisol in saliva (live pigs), serum and urine, and creatine kinase in serum after slaughter.

The main objective of the study was to determine the effect of sex and time to slaughter (transport and lairage duration) on pork quality in relation to cortisol and creatine kinase levels in crossbred pigs. In Chapter 3, sex and time to slaughter (transport and lairage duration) effects on the levels of cortisol and creatine kinase was determined. The effect of lairage duration and its interaction with sex on pork quality traits (pH, colour, cooking loss and tenderness) was determined in Chapter 4. This study was done following normal routine of farm to abattoir conditions and using the same breed (crossbreeds) and same age (six months old) pigs.

As shown in Chapter 3, cortisol response to time to slaughter (transport and lairage duration), and levels of creatine kinase between sexes clearly indicated that pre-slaughter handling in pigs is a stressful experience. Gilts were least responsive to psychological stress compared to

boars, as shown by decreased saliva cortisol levels after transportation and decreased serum and urine cortisol levels during lairage duration. This was attributed to the fact that boars tend to have an aggressive temperament and are highly excitable compared to gilts (Faucitano and Schaefer, 2008). However, reaction to physical strain or contact between gilts and boars was more evident in the former, as was shown by them having higher levels of serum creatine kinase. Besides the significant correlation between cortisol saliva after transportation and urine cortisol, there was no correlation for any other cortisol measurements. As regards the functional properties of the pork in Chapter 4, samples from the carcasses of gilts had a low lightness (L*) colour component than boars. For other meat quality traits (pH, thawing loss, cooking loss and Warner-Bratzler Shear Force) from gilts and boars, there were no significant effects of pre-slaughter human-animal interactions (lairage duration). This may be attributable to the Duroc breed used as a paternal parent in the crossbreeding process which has good traits (*viz*. resistance to stress and ease of adaptation) that contribute vital production parameters when crossed with other breeds (Smiecińska *et al*., 2011). Baseline saliva cortisol had a significant correlation with meat lightness (L*). The significant correlation between saliva cortisol after transportation and meat cooking loss can probably be due to the activated ionic activity of muscle to retain natural fluids. This is supported by Daszkiewicz *et al*. (2009) who reported that at high pH_u (>5.8), the physical state of proteins will associate with more water and lower water loss during thermal treatment. Correlation analysis also showed a significant relationship between saliva cortisol after lairage duration, cortisol levels in serum and urine and meat pH_{45} and cooking loss. This may be attributable to the intensity of glycogenolysis and gluconeogenesis by cortisol in response to stressful stimuli (Hoffman and Laubscher, 2011; Peres *et al*., 2014).

5.2. **Conclusions, Recommendations and Future research**

Based on the reported saliva cortisol response to transportation, it can be concluded that psychologically; boars were more reactive than gilts in situations where there could be a threat to physical or mental homeostasis. From the pig welfare point of view, the decreased levels of saliva cortisol after transportation and serum and urine cortisol levels in response to lairage duration could suggest that lairage is necessary to recover from the previously encountered stress. The positive correlation between saliva cortisol after transportation and urine cortisol gave the impression that measuring saliva and urine cortisol levels in pigs may be useful in detecting responses of the Hypothalamic-Pituitary-Adrenal axis and Sympathetic Nervous System following acute stress. However, with regards to physiological stress, handling by sex warrants further investigation as sex mixing might have a negative effect on serum creatine kinase responses to physical strain between sexes, as shown by its (CK) high activity in gilts. On the assessment of meat colour, sex effect on meat lightness warrants further investigation to determine whether differences in the L* value are the results of suspected differences in lipid accumulation between sexes. Correlation analysis showed a significant relationship between saliva cortisol and meat lightness (L*), and saliva cortisol after treatments, serum cortisol and cooking loss. The current study's findings suggest that high levels of cortisol and creatine kinase, and decreased meat colour lightness in gilts and boars following transportation and lairage duration could be related to negative handling experiences by pigs. However, future research is also needed to evaluate various transportation times and lairage periods to determine their effects on pork quality.

5.3. **References**

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