A comparison of meat quality attributes in free-range bred pigs finished in shed or shelter systems.

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**Declaration**

This thesis has been composed by myself and has not been accepted in any previous application for a degree. The work of which this is a record, has been done by myself and all sources of information have been cited.

Amy Suckling
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Chapter 1. **Review of the Literature**

### 1.1 Introduction

Current inconsistencies in Australian pork meat quality have been largely attributed to the genetic selection for performance-based attributes such as decreased fat depth, increased lean yield and improved feed efficiency. These traits, however, have led to a product that has improved little in its quality characteristics over the last two decades (Ngapo & Gariepy, 2008). Pork meat quality has become more important due to an astute consumer, whose buying power dictates the profitability of the modern pork industry (Cassens, 2000). Australian consumers are becoming increasingly selective and less willing to accept substandard pork, many opting for red meat over pork due to previous unsatisfactory experiences. This consumer shift in attitude has recently caused a switch in focus for producers and processors to be more mindful of quality consistency (Warriss, Brown & Paściak, 2006). A major cause of inconsistent pork quality is the variation in the pH of fresh pork, often resulting in poor quality through the prevalence of pale soft and exudative (PSE) meat (Shen et al., 2006). Known for its aberrant features, PSE reduces marketability and meat processing abilities and has been reported as costing the USA pork industry, for example, $100 million per annum (Shen et al., 2006; Scheffler & Gerrard, 2007). Despite extensive research into the causes of poor quality pork, little improvement has been made; within the USA pork industry again, for example, surveys recorded the incidence of PSE in 2003 as 15.5% demonstrating minimal improvement in comparison to surveys undertaken in 1963 where the incidence of PSE was recorded at 18% (Cassens, 2000; Scheffler & Gerrard, 2007). This suggests that more work needs to be undertaken in order to further understand the inconsistency associated with pork meat quality.

The level of stored glycogen in muscle has an impact on meat quality when glycogen undergoes glycogenolysis, glycolysis and lactic acid fermentation post-mortem (Shen et al., 2006; Choe et al., 2008). Different levels of accumulated lactate and hydrogen ions from the conversion of glycogen to lactic acid is one of the main causes of variation in meat quality attributes due to its affect on muscle pH (Ryu & Kim, 2006). The muscle glycogen store and the post-mortem processes of anaerobic glycolysis and fermentation are directly affected by
many external and predisposing factors such as genetics, feed intake, animal handling, post-slaughter chilling rate and production system (Van der Wal, Engel & Hulsegge, 1997; Terlouw, Berne & Astruc, 2009). It is due to these many factors and the difficulty scientists and processors have in controlling them, that the results of numerous studies have been highly variable (Rosenvold & Andersen, 2003). This literature review will attempt to clarify discrepancies in the literature and underpin the current understanding of the biochemistry of meat quality and the pre- and post-slaughter factors that affect them.

1.2 Pork Quality

Pork quality refers to the measurable technical muscle quality characteristics of muscle glycogen content, colour, water holding capacity (WHC) and pH, although other characteristics such as fat composition, nutritional value, yield and eating quality are also frequently referred to in the literature. The mechanisms behind the development of certain pork quality attributes have been explored in some detail over the last 50 years (Briskey & Wismer-Pedersen, 1961; Briskey, 1964; Briskey et al., 1966; Greaser et al., 1969), and a strong understanding of the biochemical conversion of living muscle tissue into meat has been established (Scheffler & Gerrard, 2007). Typically, four scientific quality classes that take into account colour, texture and WHC can define pork meat. These classes are pale soft and exudative (PSE) and dark firm and dry (DFD), and the lesser known groups reddish-pink soft and exudative (RSE) and reddish-pink firm and non-exudative (RFN) (Moya et al., 2001; Qiao et al., 2007). These quality classes are frequently defined by technological meat quality attributes, which are ultimately dependent on the extent of pre- and post-slaughter metabolism within pig muscle tissue.

1.2.1 Mechanisms of muscle metabolism

The metabolic processes that occur within the muscle tissue of an animal before, during and after slaughter contribute to the development of its meat quality attributes (Scheffler & Gerrard, 2007). In order to understand the differences in meat quality a thorough understanding of the metabolic processes involved is necessary.
1.2.1.1 Pre-Slaughter metabolism

Muscle tissue is able to maintain homeostasis by modulating its metabolic activity in response to changes in energy expenditure and storage (Scheffler & Gerrard, 2007). The metabolism of muscle cells generates energy in the form of adenosine triphosphate (ATP), which is supplied by fatty acids from adipose tissue, ketone bodies from the liver, glucose from the blood or stored glycogen and phosphocreatine in the muscle (Lehninger, Nelson & Cox, 2005). The energy required for the majority of an animals muscular functions is regulated by aerobic metabolism, utilizing fatty acids, ketone bodies and blood glucose in the presence of oxygen to enable low level muscular contraction, calcium sequestration and ion gradient regulation (Scheffler & Gerrard, 2007).

In the instance of high feed intake and rest, blood glucose levels are high and ample ATP is being supplied to muscle tissue; glycogen is synthesised by glycogenesis and stored in the muscle until the energy is required (Choe et al., 2009). Muscle glycogen stores are utilised by the muscle by the mechanisms of glycogenolysis, and then metabolized through glycolysis (Lehninger, Nelson & Cox, 2005). In anoxic conditions this leads to the formation of lactate, which reduces the pH of the muscles and decreases their efficacy when high intensity contraction continues over an extended period (Lehninger, Nelson & Cox, 2005). In normal physiological conditions, lactate will be removed from the muscle by the circulatory system for reutilisation by the Cori cycle in the liver. Here, it is reconverted to pyruvate and then glucose by gluconeogenesis (Lehninger, Nelson & Cox, 2005).

1.2.1.2 Post-Slaughter metabolism

Post-slaughter muscle metabolism reflects the anaerobic pathways that occur in living muscle tissue as described previously, with one major exception being that metabolic by-products are unable to be removed from muscle tissue by the blood stream post-mortem (Scheffler & Gerrard, 2007; Choe et al., 2008). The absence of blood flow post-mortem prevents the Cori-cycle from occurring and anaerobic metabolism continues despite this in an attempt to maintain muscle homeostasis and delay the onset of rigor mortis. This causes lactic acid to accumulate causing the muscle pH to decline until either muscle glycogen is exhausted or glycolytic, glycolytic or fermentative enzymes are inactivated (Pösö & Puolanne, 2005; Choe et al., 2008). The lactic acid accumulation and heat generation caused by post-mortem anaerobic metabolism, in combination with a retarded chilling rate, can lead to the
denaturation of muscle proteins including enzymes used in metabolic processes (Scheffler & Gerrard, 2007). This inevitably leads to a slowing down of anaerobic metabolism and can halt these processes before glycogen stores are used up, thus the glycogen content of muscle tissue pre-slaughter can typically determine the pH of pork.

When ATP supplies from anaerobic glycolysis are near exhaustion in post-mortem muscle tissue and are no longer being replenished by muscle glycogen stores, crossbridges begin to form between contractile actomyosin muscle fibres. This leads to the shortening of muscle sarcomeres and causes muscles to stiffen, a characteristic of rigor mortis (Scheffler & Gerrard, 2007). The tensed muscle protein fibres eventually degrade post-mortem, muscle fibres relax and the rigidity ceases as the muscles lose their structural integrity (Pösö & Puolanne, 2005). It is the combined effects of protein denaturation and pH decline due to the metabolism of muscle glycogen post-mortem that result in the conversion of living muscle tissue to the final pork product.

1.2.1.3 Technical meat quality
Technical meat quality attributes enable the quantitative comparison of meat quality traits between carcasses. They also enable educated assumptions to be made about their metabolic development based on combined effects of glycolytic potential, pH, colour and WHC.

1.2.1.3.1 Glycolytic potential and pH
In the conversion of muscle to meat, pH plays an intrinsic role in determining its quality (Bendall & Swatland, 1988). Additionally, the rate of pH decline also contributes to its pork quality attributes (Scheffler & Gerrard, 2007). While the complex interactions of pre-slaughter and post-slaughter metabolism are what ultimately determine this rate of pH decline, the potential an animal has to develop a particular muscle pH is based solely on its glycolytic potential (Van Laack & Kauffman, 1999; Hamilton et al., 2002). The glycolytic potential of an animal is largely its energy status, and in pork meat quality, the focus is on the level of stored muscle glycogen immediately pre-slaughter. The exhaustion of muscle glycogen stores prior to stunning reduces the glycolytic potential an animal has and directly limits the amount of lactic acid that can be produced post-mortem (Scheffler & Gerrard, 2007). Hence, an animal with low glycolytic potential will produce meat with a high pH. Conversely, an animal with higher glycolytic potential has more glycogen stored in muscle
tissue and has a greater capacity to produce low pH pork (Rosenvold & Andersen, 2003). However, this is not always the case, an animal with a high glycolytic potential will not always produce a high level of lactic acid post-mortem. It is due to the complex nature of pre- and post-slaughter metabolism and the multiple factors that affect it that lead to the four pork quality classes described.

1.2.1.3.2 Proglycogen and Macroglycogen
The importance of glycogen in the formation of pork quality attributes is undisputed, however, limited work has been undertaken to investigate the impacts of different forms of glycogen. A greater understanding of how proglycogen and macroglycogen forms are utilised in pig muscle could improve future approaches towards pork meat quality. Proglycogen is a small (400kDa) acid insoluble glycogen molecule that has a lower carbohydrate to protein ratio than its larger counterpart macroglycogen. In the living animal proglycogen is favourably synthesised from dietary energy post-exercise and is also preferentially degraded in favour of macroglycogen in anaerobic metabolism post-mortem (Sterten et al., 2010). Conversely, macroglycogen is acid soluble, has a higher ratio of carbohydrate to protein and is also larger in size (upto $10^4$ kDa) than proglycogen (Rosenvold & Andersen, 2003). When pre-slaughter muscle glycogen levels are high, the concentration of macroglycogen increases (Sterten et al., 2010). Rosenvold, Essén-Gustavsson, & Andersen (2003) found that the reduction in total muscle glycogen stores caused by extended dietary manipulation is actually a reduction in macroglycogen and that subsequent reduced post-mortem glycolysis is the consequence of the reduced metabolism of the proglycogen pool. This indicates that the predominant glycogen type stored in an animal, may help determine the rate of glycogen converted to lactate post-mortem and contribute to its meat quality attributes.

1.2.1.3.3 Technical meat quality measures and pH
While pH and glycogen are considered technical meat quality measurements these typically influence the extrinsic appearance and structure of the meat resulting in differing meat quality aspects. As discussed the level of glycogen pre-slaughter affects the pH post-slaughter, and in turn this pH, the rate of decline and temperature effects the other technological meat quality measurements. Normally, the pH of living pork muscle is around 7.2 and drops to 5.5-5.8 within 24 hours post-slaughter (Brewer et al., 2001). The pH of pork meat is usually measured at 45-60 minutes post-mortem, to give an indication of the rate of pH decline and is
referred to as early post-mortem pH (Scheffler & Gerrard, 2007). Additionally, the ultimate pH (pHu) is measured 24-48 hours post-mortem to establish the extent of the pH decline and is considered low below 5.4 and high above 6 (Brewer et al., 2001). The pHu in particular, correlates with the meat quality attributes of colour and WHC.

1.2.1.3.3.1 Colour
The colour of pork affects the consumer appeal of the raw meat product, with both very pale and very dark meat colours being less acceptable to consumers (Bredahl, Grunert, & Fertin, 1998). Pork colour is measured quantitatively using a chroma meter which provides values for reflectance $L^*$ values for lightness, $a$ values for red-green reflectance and $b$ values for yellow-blue reflectance (Brewer et al., 2001). The $L^*$ value is the most commonly used measure and the value increases as meat colour gets lighter. Pork colour is correlated to pHu, with high and low pHu producing darker and lighter meat colour respectively (Huff-Lonergan et al., 2002). This is attributed to the reduced solubility of sarcoplasmic proteins as pork pH decreases post-mortem; the more sarcoplasmic proteins precipitate the lighter meat colour becomes (Bredahl, Grunert, & Fertin, 1998). This suggests that pork with dark meat colour and high pHu has little or no sarcoplasmic protein precipitation post-mortem.

1.2.1.3.3.2 Water Holding Capacity
WHC is used as a determinant of meat quality due to its effect on consumer appeal and its correlation with pHu (Aaslyng et al., 2003). There is also some evidence to suggest that WHC is related to the juiciness of pork during chewing and is therefore implicated in pork eating quality (Van Oeckel, Warnants & Boucqué, 1999; Aaslyng et al., 2003). Pork with a low WHC is unappealing to consumers due to the visual appearance of liquid exudate (Scheffler & Gerrard, 2007). Conversely, very high WHC is unappealing due to its dry tacky nature (Scheffler & Gerrard, 2007). WHC is commonly measured in two ways, drip loss percentage and filter paper absorption methods and is often represented as a percentage drip loss value (Van Oeckel, Warnants & Boucqué, 1999). Drip loss is measured as a percentage of weight lost due to water exudation with the higher the percentage the lower the WHC of the meat (Van Oeckel, Warnants & Boucqué, 1999). Filter paper methods are recorded as filter paper weight gained or visual moisture score of exudate absorption (Van Oeckel, Warnants &
Boucqué, 1999). Major factors affecting the WHC of pork include temperature and pH post-mortem which determine the extent of myofibrillar protein denaturation and myofibrillar net charge (Schäfer et al., 2002). High temperatures and low pH are associated with high levels of protein denaturation, whilst extended pH decline is associated with a reduced net charge of myofibrils (Schäfer et al., 2002; Scheffler & Gerrard, 2007). As the myofibrillar proteins denature, a reduction in myosin head length within the muscle fibres draws thick and thin filaments closer together, leading to the expulsion of water (Schafer et al., 2002). Alternatively, when the net charge of myofibrils has been reduced, myofilaments are attracted toward each other forcing water out of the muscle fibre lattice (Scheffler & Gerrard, 2007). Both ultimately lead to an increase in drip loss and reduce the WHC of pork.

1.2.2 Pork quality classes

1.2.2.1 Reddish-pink, firm and non-exudative (RFN)
Pork classified as RFN has an ideal WHC, colour and pH and is regarded, quality-wise, as normal. Technical pork quality attributes for RFN have been defined as having a drip loss less than or equal to 5%, a hunter $L^*$ lightness score of between 42 – 50 and an pHu of less than 6 (Warner, Kauffman & Greaser, 1997; Kauffman et al., 1998;). The metabolic processes involved in the production of these qualities are a gradual rate of glycolysis leading to moderate lactic acid production and moderate levels of protein denaturation (Faucitano et al., 2010b). These traits are considered ideal due to their positive influence on eating quality and preferable appearance as a raw meat product. However, this is not always prominent and has been shown to occur as little as 13% in some quality audits (Faucitano et al., 2010b), highlighting this decline in product quality.

1.2.2.2 Pale, soft and exudative (PSE)
PSE pork has been well described in the literature due to its negative qualities and higher prevalence in industry than other poor quality pork classes. The subjective characteristics of PSE pork include a distinctly pale meat colour with a shiny wet appearance and soft spongy texture (Owen et al., 1999). The meat’s unappealing appearance affects consumer acceptability of raw meat and its quality characteristics make PSE pork difficult to use in value adding processes such as moisture infusion (Shen et al., 2006; Warriss, Brown & Paściak, 2006; Scheffler & Gerrard, 2007). Technical pork quality attributes of PSE have
been identified and although variations between texts exist, the common classifications have a lightness score of $L^*$ greater than 50, a drip loss of greater than 6%, a pH two hours post-mortem of less than 5.8 and an pHu between 5.3 and 5.7 (Toldra & Flores, 2000; Ryu & Kim, 2006).

It has been widely reported that PSE pork’s unpleasant form, processing issues and poor eating quality, are largely due to an accumulation of lactic acid at high muscle temperatures early post-mortem (Shen et al., 2006). The typical metabolic profile of PSE meat involves rapid early post-mortem glycolysis which is signified by the development of a pH below 6 within the first hour post-slaughter (Scheffler & Gerrard, 2007). Additionally, Briskey & Wismer-Pedersen (1961) found that pigs that develop PSE pork are more likely to have a high concentration of proglycogen and total glycogen 45 -60 minutes post-slaughter than non-PSE pigs, coinciding with the rapid pH decline. It is through the study of PSE meat that the rate and extent of the pH decline immediately post-exsanguination has been identified as a critical factor affecting pork quality (Briskey et al., 1966; Cassens, 2000).

1.2.2.3 Dark, firm and dry (DFD)

Converse to PSE pork, DFD pork is characterised by visually darker meat which is dry and firm to the touch due to an increased WHC (Van de Perre et al., 2010). The technological pork quality attributes that characterise DFD are a high pHu greater than 6, a drip loss of less than 2% and hunter $L^*$ values for lightness less than 43 (Joo et al., 1999; Lee et al., 2000; Guàrdia et al., 2005; Ryu & Kim, 2006). DFD pork is of concern to producers due to the increased propensity for microbial spoilage and poor dry curing properties (Guàrdia et al., 2005). A further problem for processors is the identification that the dry appearance of meat, as found in DFD pork, is less appealing and less likely to be purchased by consumers (Brewer & McKeith, 1999). Additionally, 10% of leg ham pork has been identified as being DFD, in a national survey undertaken in the USA in 1992; implicating it as an important meat quality issue to the industry (Kauffman et al., 2000). The DFD characteristics can be attributed to a high pHu, which is the result of a reduced glycolytic potential pre-slaughter leading to limited lactic acid production during post-mortem metabolism. This lower glycolytic potential limits the concentration of glucose and glycolytic intermediates and forces microorganisms to utilise proteins as an energy source resulting in faster muscle decomposition and unappealing odours (Faucitano et al., 2010b). The literature largely attributes low glycolytic potential pre-
slaughter to periods of extended activity or chronic stress (Van de Perre et al., 2010). Associated pre-slaughter factors that may impact on the incidence of DFD include trucking conditions, season, gender, transport stocking density, fasting time and estimated carcass lean (Guàrdia et al., 2005). Management of these factors are important to reduce the occurrence of DFD pork.

### 1.2.2.4 Reddish-pink, soft and exudative

Reddish-pink, soft and exudative pork (RSE) is a pork quality group only introduced in the literature over the last two decades, however, reports suggest that the incidence of RSE pork is as high as 30% in Canada indicating it as a major quality defect (Van de Perre et al., 2010). The characteristics of RSE pork are similar to those of PSE except the colour is closer to that of normal RFN pork, and it is the soft and exudative nature of RSE pork that makes it undesirable to processors and consumers. Toldra & Flores (2000) describe RSE as having a lightness score of \( L^* \) 44-50, a drip loss of greater than 6% and a 2 hour post-mortem pH of less than 5.8. Other studies describe RSE pork as having a higher pHu than PSE meat due to a lower pre-slaughter glycolytic potential (which is higher than RFN pork) and limited protein denaturation, meaning the cause of the soft and exudative characteristics remain largely unknown (Faucitano et al., 2010b). Previous suggestions that RSE may be related to the prevalence of the halothane gene have been refuted by Cheah, Cheah & Just (1998), who claim that RSE will continue to persist even with the eradication of the halothane gene. Additionally, the RN* gene described later in further detail, has also been eliminated as a potential cause of RSE pork by Van Laack & Kauffman (1999). Whilst the high prevalence of RSE pork has been established, the economic impact of this newly identified pork quality class needs to be assessed in order to establish whether or not it is a concern that needs to be addressed by producers in future.

### 1.3 Animal predispositions to meat quality traits

The genes an animal carries define what possible phenotypes it expresses. It is phenotypes such as percentage lean, back fat and feed efficiency that have been selected for in pigs to improve productivity. Additionally, the sex of an animal also plays a role in determining meat quality traits due to differences in the utilisation of energy. Meat quality traits can also be selected for and the inclusion of such in breeding programs can benefit producers and processors by ensuring product quality. Similarly, particular genes can also negatively impact
on pork quality and the halothane gene and RN\textsuperscript{-} genes are two genes that have had significant negative implications on muscle metabolism, technological meat quality characteristics and consumer appeal.

1.3.1 Halothane gene
The halothane gene causes malignant hyperthermia or porcine stress syndrome in pigs. This causes stress hypersensitivity leading to an escalated rate of post-mortem glycolysis, which subsequently increases the incidence of PSE carcasses (Jeremiah et al., 1999; Channon, Payne & Warner, 2000; Rosenvold & Andersen, 2003). It is the result of a causative mutation of the ryanodine receptor isoform (RYR1) and pigs are affected whether they are homozygous or heterozygous for the gene, however, heterozygotes are less affected (Rosenvold & Andersen, 2003; Van de Perre et al., 2010). Those animals carrying the gene are known to have an increased carcass yield, increased feed efficiency and increased lean percentage which are traits heavily selected for in the pork industry (Channon, Payne & Warner, 2000). This selection is what ultimately may have led to the dramatic increase in carriers of the halothane gene by the early 1990s. Since its identification as a cause of pork quality problems efforts have been made to eradicate the gene from commercial herds, and today the impacts of the halothane gene may no longer be of consequence to the pork industry (Rosenvold & Andersen, 2003). Although the halothane gene has a significant impact on meat quality, the many meat quality studies undertaken whilst the halothane gene was present in commercial herds may have little application in today’s halothane free herd. Results from studies where pigs with the halothane gene remained unidentified have the potential to be misleading.

1.3.2 RN\textsuperscript{-} gene
The RN\textsuperscript{-} gene is a dominant gene commonly associated with the commercially used Hampshire breed, and is the result of a causative mutation in the PRKAG3 gene which encodes for muscle specific isoform of the regulatory subunit of adenosine monophosphate-activated protein kinase (Lindahl et al., 2004; Rosenvold & Andersen, 2003). RN\textsuperscript{-} carriers have high levels of muscle glycogen pre-slaughter but unlike the halothane gene, the RN\textsuperscript{-} gene does not impact early post-mortem glycolysis and instead causes a normal early post-mortem pH and a very low pHu often referred to as ‘acid meat’ (Van Laack & Kauffman, 1999). This can be distinguished from PSE by its lower levels of protein denaturation and normal meat colour (Van Laack & Kauffman, 1999; Rosenvold & Andersen, 2003).
Similarities to halothane gene carriers include a reduced WHC and carcasses with a lighter meat colour caused by the denaturation and precipitation of sarcoplasmic proteins at low pH (Hamilton et al., 2000). This gene is also associated with leaner carcasses, reduced technological yield, high muscle glycogen stores pre-slaughter and extended pH decline post-mortem (Le Roy et al., 1990; Milan et al., 1996). Interestingly, higher levels of glycogen pre-slaughter in RN pigs is the result of high macroglycogen pools, suggesting that macroglycogen may be the reason RN pigs do not experience rapid early post-mortem glycolysis or develop PSE characteristics. This is due to the preferential metabolism of proglycogen stores over macroglycogen stores early post-mortem (Rosenvold & Andersen, 2003). Although the RN gene carriers produce suboptimal technological meat quality traits, the commercial implications of the RN gene are yet to be quantified, as the consumer response to ‘acid meat’ has yet to be established and the economic impacts of a scientifically demonstrated reduction in technological yield is only theoretical at this stage.

1.3.3 Polygenic effects

Many selectable meat quality traits are not the result of a single gene but rather a combination of multiple genes at different locations in the pig genome. These traits are referred to as polygenic and as a result many have low to moderate heritability. Heritability denotes the proportion of variation between individuals in a population that is influenced by genetic factors and is represented as a decimal between 0 and 1, a value of 1 infers that all variation is attributed to genetic factors and a value of 0 means that none of the variation is due to genetic factors. Oksbjerg et al. (2000) details the heritability of glycogen storage in Landrace, Yorkshire/Large White and Duroc boars as 0.37 which represents a moderate level of heritability. However, Rosenvold and Andersen (2003) speculated that the observed heritability of glycogen is partly related to newly described alleles on the PRKAG3 gene, the mutation of which is the cause of RN affected pork. This was confirmed by Lindahl et al. (2004), who discovered that four allele combinations or haplotypes are often present in commercial slaughter lines of Landrace, Large White, Duroc, Duroc synthetic and Berkshire swine and affect pork muscle glycogen content, pHu and meat colour (Ciobanu et al., 2001). Historically, the selection of traits for improved performance in pigs such as lean carcass yield and growth rate, has led to poorer meat quality attributes such as paler, less red meat colour (Oksbjerg et al., 2000), lower early post-mortem pH and reduced WHC (Huff-Lonergan et al., 2002). Moreover, the majority of technological meat quality attributes are
only low to moderate in heritability usually falling between 0.15-0.30 with pHu having a range between 0.07 and 0.39 whilst post-mortem pigment content demonstrates a high heritability of 0.65 (Rosenvold & Andersen, 2003). However, other non-technological meat quality traits exist with higher levels of heritability such as intramuscular fat (0.40 to 0.50) and can be selected for to improve pork eating quality and consumer acceptability (Rosenvold & Andersen, 2003).

1.3.4 Breed effects
Just as particular breeds of pigs are known to have a high lean yield, others also have a reputation for superior eating quality. As a result, particular breeds are often incorporated into commercial herds to improve the eating quality of pork, the most commonly described breeds include the Tamworth, Berkshire, Hampshire, Duroc and Chinese purebreds (D’Souza & Mullan, 2002; Ngapo & Gariepy, 2008). Certain breeds also have higher incidences of the halothane and RN⁻ genes, which can negatively affect technological meat quality attributes. The Pietran, Poland China and Landrace breeds are known for their association with the halothane gene, whilst the RN⁻ gene is common in the Hampshire breed (Rosenvold & Andersen, 2003). The Duroc breed is commonly introduced into the terminal line to improve meat quality as described by D’Souza and Mullan (2002) and Ngapo & Gariepy (2008). Both concluded that in Large White and Landrace based system, pigs containing 50% Duroc had meat with a higher pHu, lower WHC and higher intramuscular fat than 25% Duroc pigs. Channon, Kerr & Walker (2004) also concluded that 100% Duroc pigs were juicier than 50% and 0% Duroc pigs, demonstrating the impact of breed on meat quality attributes.

1.3.5 Gender
Technological pork quality attributes differ between entire male pigs, barrows and female pigs. Entire male pigs have a higher early post-mortem temperature in the m. longissimus lumborum muscle and a higher pHu when compared to female pigs (Van der Wal, Engel & Hulsegge, 1997). Additionally, female pigs have a lower pHu and increased surface exudate than barrows and male immunological castrates (D’Souza & Mullan, 2002). Smaller trends in hunter *L values and WHC were also seen by Van der Wal, Engel & Hulsegge (1997) and this could be confirmed by Channon, Kerr & Walker (2004), who determined that entire males produce darker pork. It has also been shown that entire males have tougher pork than female pigs who have tougher pork than immunological and surgical barrows (Channon,
Furthermore, female pigs demonstrate characteristic tendencies towards PSE pork whilst male pigs have technical meat quality characteristics representative of meat at the DFD end of the scale.

1.4 Pre-Slaughter Factors

Pre-slaughter factors include all the aspects of activity, nutrient intake, season and handling that affect a pig throughout its life prior to slaughter. These factors are difficult to keep consistent and cause variations in meat quality from day to day contributing to what is known as the day effect. The glycolytic potential of a pig is affected by pre-slaughter factors, which as previously described, impacts on its muscle metabolism and technological meat quality.

1.4.1 Climate and Season

Meat quality attributes are known to vary between the seasons of winter and summer. Summer weather is associated with a higher incidence of PSE meat whilst winter is associated with a higher incidence of DFD meat (O’Neill et al., 2003; Guàrdia et al., 2005). Van de Perre et al., (2010) determined that in systems where temperature was not regulated, DFD was more likely to occur during the colder winter months when pigs are more likely to use up energy reserves to shiver or group together in an attempt maintain core body temperature. The same study suggested that the increased incidence of PSE meat in summer months was likely due to a lack of sweat glands, increasing sensitivity to high temperatures and stress (Van de Perre et al., 2010). This would lead to the animal being stunned whilst the body temperature was high and anaerobic metabolism continuing post-slaughter, resulting in rapid lactate production early post-mortem, a high carcass temperature and protein denaturation as seen in PSE pork (Van der Wal, Engel & Hulsegge, 1997). This suggests that season and climate play important roles in determining pork quality characteristics.

1.4.2 Production system

Studies suggest that modern consumers perceive welfare friendly production systems as nutritionally superior, safer and more palatable (Lebret et al., 2002; Edwards, 2005; Bonneau & Lebret, 2010). This consumer attitude towards welfare friendly and alternative housing systems has motivated the pork industry to include alternatives to raising and finishing pigs in conventional intensive sheds. Some of the popularly introduced systems include outdoor free-
range, hoop barn/eco-shelter and enriched indoor systems. The implications of these systems in comparison to conventional sheds on the eating quality of pork products has yet to be understood. Whilst the fat composition has been thoroughly investigated with regards to free-range systems, limited thought has been given to the impacts on technological meat quality attributes.

1.4.2.1 Free-range production

In Australia, free-range pigs are defined as those that have been bred and permanently kept in outdoor paddocks for the entirety of their lives with free access to shelter (Australian Pork Limited, 2012). Generally, well fed free-range pigs should have a higher glycogen store, lower pHu and decreasing technological yield due to the increased frequency of exercise (Bee, Guex & Herzog, 2004; Bonneau & Lebret, 2010). However, this is an area that lacks clarity as numerous conflicting results have been reported on eating quality and technical meat quality attributes in outdoor raising systems (Lebret et al., 2002; C. Terlouw et al., 2009). Similar studies report no difference in juiciness (Gentry et al., 2002) or decreased juiciness (Enfält et al., 1997) between studies in pigs raised in similar outdoor conditions compared to conventionally raised pigs. Others report that pHu and early post-mortem pH do not differ between outdoor raised and conventionally raised pigs (Gentry et al., 2002; Lebret et al., 2002), whilst others reported lower early post-mortem pH, lower pHu and reduced WHC (Enfält et al., 1997; Sather et al., 1997). Further adding to the uncertainty, Lambooij et al., (2004) observed higher pH and lower lactate levels early post-mortem in free-range pigs when compared to conventionally raised pigs which were more exudative, had a rapid early post-mortem pH decline and higher pHu. This study suggests that pigs raised conventionally are more likely to develop PSE qualities and potentially poorer meat quality than free-range pigs. However, differences exist in other pre-slaughter factors between these studies that could explain the variation in results. The most common was of free-range conditions particularly stocking density which ranged from 1.25m²/animal (Lambooij et al., 2004) to 980m²/animal suggesting that increased stocking densities could lessen the potential for PSE development (Enfält et al., 1997). The unstandardized nature of free-range raising environments makes the comparison of meat quality studies very difficult, and furthermore, the number of factors that affect a pig’s glycolytic potential and resulting meat quality makes regulating the meat quality of free-range pork problematic.
1.4.2.2 Eco-shelters & hoop barns

Eco-shelters or hoop barns are simply constructed semi-outdoor sheds with canvas or tarpaulin pulled tightly over arch shaped metal pipes acting as the roof to a walled concrete base (Honeyman, 2005). They were originally designed to reduce production costs and are filled with straw or similar bedding material to support group-housing conditions (Patton et al., 2008). However, due to their semi-outdoor design and floor bedding, they have become an easy and cost effective method of improving consumer perceptions of pig welfare (Van de Weerd & Day, 2009). Limited work has been undertaken to establish what effects, if any, this type of system has on the meat quality attributes of pigs. Van de Weerd & Day (2009) explained that straw bedding is a useful enrichment tool that encourages foraging behaviours and pig welfare. However, Patton et al. (2008) established that pigs raised with straw bedding had higher energy demands for growth and consequently required more feed. This study also determined that there was no difference in pH, WHC, colour or firmness in pork between hoop raised and standard shedded pigs. This is supported by Honeyman (2005), who determined that product quality differences were minimal in pigs housed in hoop barns when compared to conventionally housed pigs. This was attributed to the enhanced ability of pigs to compensate to changes in environmental temperature when housed as a group with bedding. Van de Weerd & Day (2009) later described the literature on straw based systems and determined the effect on meat quality as inconsistent and a number of conflicting results were produced. The conflicting nature of results suggests that the pork product produced from outdoor and semi-outdoor systems such as eco-shelters will be difficult to control and often vary between producers and processors. Nevertheless, the increased energy demands of pigs in straw based systems suggests that a lower glycolytic potential is more likely in pigs housed in eco-shelters suggesting a propensity towards the development of DFD characteristics.

1.4.3 Fasting

It is generally common practice to fast pigs prior to slaughter in an aim to prevent microbial cross contamination during slaughter and improve pig welfare during transport (Faucitano et al., 2010a). Fasting has also been tested as a means to reduce muscle glycogen stores pre-slaughter in an aim to increase the pHu, improve WHC and colour thereby preventing PSE meat (Warriss, 1982; Rosenvold & Andersen, 2003). However, according to Eikelenboom, Bolink & Sybesma (1991) and Wittmann et al., (1994), the fasting pre-slaughter required to
achieve a reduction in muscle glycogen stores is greater than 24 hours which is likely to be a welfare concern.

Leheska, Wulf & Maddock, (2003) tested the effects of fasting on meat quality and included the variable of travel time pre-slaughter. When compared to non-fasted pigs, pigs fasted for 48 hours and transported for 30 minutes had a higher pHu, darker meat colour, higher marbling score and lower drip loss. However there was no effect of fasting when pigs were transported for 2.5 hours or 8 hours pre-slaughter. This suggests that extended travel may mimic the effects of fasting on muscle glycogen stores in pig tissue over extended periods.

It has also been suggested that the length of fasting alters the metabolic profile of muscle tissue post-mortem (Sterten et al., 2010). Short-term fasting of 4 hours resulted in delayed glycogen degradation and a gradual decline in pH resulting in a lower pHu when compared to long-term fasting of 26.5 hours (Sterten et al., 2010). Pigs with genotypes favouring meat quality were the most positively affected by fasting than pigs that did not have a meat quality favouring genotype. Pigs selected for meat quality attributes had a lower glycolytic potential and an increased pHu in the m. longissimus dorsi when fasted (Van der Wal, Engel & Hulsegge, 1997). This suggests that fasting may be a useful tool for preventing PSE type meat quality in pigs selected for meat quality attributes, but may not be as successful at reducing PSE in other pigs.

1.4.4 Long-term stressors
Stress in pigs is commonly associated with meat quality defects; however, long-term stressors and short-term stressors affect pre-slaughter metabolism differently and as a result have varying outcomes for meat quality. Long-term stressors include all aspects of handling that an animal is exposed prior to reaching the abattoir, and include on-farm handling, transportation and mixing of unfamiliar animals (Rosenvold & Andersen, 2003). They have a greater effect on glycogen energy storage depletion than short-term stressors, which occur upon arrival at the abattoir. As a result, long-term stressors are most commonly associated with DFD characteristics due to a reduction in glycolytic potential. A complete understanding of the impacts of long-term stressors on meat quality is important so that they can be managed appropriately.
1.4.4.1 On-farm stressors

The poor handling of an animal on farm can lead to an increased susceptibility to other pre-slaughter stressors (D’Souza et al., 1998). The reasons behind this are explored by Rushen, Taylor & de Passillé (1999) who detailed that pigs aversively handled learn to associate their negative experience with people. This learned behaviour could further enhance the stress response to handling and novel activities such as loading and transport on the day of slaughter and can have detrimental effects on production and meat quality (Grandin, 1998). Pigs susceptible to stress due to negative associations with handling are more likely to develop a rapid early post-mortem pH decline and PSE characteristics due to hasty post-mortem glycolysis caused by heightened stress levels during ushering to the stunner (D’Souza et al., 1998). Alternatively, a study undertaken by Schwartzkopf-Genswein et al. (2012) suggests that pigs handled poorly during truck loading on farm are more likely to exhaust glycogen stores during transport resulting in a higher muscle pH post-mortem and DFD characteristics.

It is evident that rough or negative handling on farm is not only a welfare concern (Grandin, 1998) but can also induce stress-hypersensitivities through learned behaviour or increase glycogen storage depletion during transport; both of which have negative effects on meat quality and should be eliminated from current handling practices.

1.4.4.2 Transportation

It has been widely reported that the exercise of loading on and off trucks during travel to the processing plant are the most stressful moments of pre-slaughter handling to pigs (Barton-Gade & Christensen, 1998). Numerous other factors affect the experience pigs have on the journey from the farm, including the quality of the vehicle, ventilation, stocking densities and travel distance (Barton-Gade & Christensen, 1998; Warriss, 2003). Leheska, Wulf & Maddock (2003) found that glycolytic potential declined with the length of transport, when tested at travel times of 8 hours, 2.5 hours and 30 minutes, resulting in an increasingly higher pHu and darker meat colour. Increasing transport time also resulted in decreased *L* score, drip loss, cooking loss and shear force values (Shen et al., 2006). Fortin (2002) observed that periods of transport lasting 50 minutes were associated with a higher incidence of PSE. Thus 50 minutes transport was insufficient to deplete muscle glycogen stores but stressful enough to cause rapid early post-mortem glycolysis. This suggests that the longer the transportation,
the more vulnerable pork was to develop DFD characteristics than shorter travel, which increased the susceptibility to develop PSE characteristics.

1.4.4.3 Mixing of pigs
Fighting behaviour during transport and lairage pre-slaughter is the reason that the mixing of unfamiliar pigs should be avoided (Guise & Penny, 1989; Warriss, 1998). Fighting often leads to reduced carcass value through the development of skin lacerations and also reduces the glycolytic potential of pigs pre-slaughter ultimately leading to higher pHu and possible DFD (Warriss & Brown, 1985; Faucitano, 1998). The early post-mortem pH remains unaffected by fighting behaviour according to Warriss & Brown (1985), therefore PSE is not an expected outcome of fighting animals.

1.4.5 Short-term stressors
Short-term stressors are those aspects of animal handling that occur upon arrival at the abattoir, and are more likely to affect metabolic rate and body temperature immediately pre-slaughter than long-term stressors. These stressors include time and handling in lairage as well as stunning processes (Rosenvold & Andersen, 2003). These are associated with PSE meat due to their role in causing acute stress, which leads to the production of reactive oxygen species (Barbut et al., 2008). This process will not be detailed here, however, this is believed to initiate the cascade of events leading to rapid anaerobic glycolysis, elevated muscle temperature and rapid pH decline immediately pre-slaughter (Barbut et al., 2008). The association of short-term stressors with acute stress and the formation of PSE meat make management of these important.

1.4.5.1 Lairage
Although transport has been demonstrated as a factor affecting meat quality, Van der Wal, Engel & Hulsegge (1997) ascertained that time and handling in lairage were much more important factors than transport distance, fighting, driver and truck variation. In fact, time and handling in lairage were such important factors that the impact of stress experiences during transport the morning of slaughter was statistically undetectable (Van der Wal, Engel & Hulsegge, 1997). Resting period had significant impacts on muscle temperature 45 minutes post-mortem, pHu and meat colour with optimal resting times calculated to be 3 hours 25
minutes for m. *longissimus lumborum* and four hours 14 minutes for the m. *Semimembranosus*. This is supported by studies by Warriss *et al.* (1995) who established general standards for rest time to be between two and four hours pre-slaughter. Furthermore, Van der Wal, Engel & Hulsegge (1997) determined that as the resting time in lairage increased, so did pHu resulting in darker meat colour. Eikelenboom, Bolink & Sybesma (1991) indicated that slaughter immediately upon arrival to the slaughterhouse causes an increased incidence of PSE. Equally, extended time in lairage can lead to recurrence of fighting behaviours and DFD (Nanni Costa *et al.*, 2002), however this may also be due to the depletion of glycogen stores as a result of fasting (Sterten *et al.*, 2010). Despite these findings, optimal resting time in lairage depends on: conditions in lairage, mixing of unfamiliar animals, and travel stress. When these three aspects of pre-slaughter handling are moderated to low stress handling, pork quality has been found to be independent of lairage time altogether (Dall Aaslyng & Barton-Gade, 2001).

1.4.5.2 **Stunning**

Electrical current and carbon dioxide gassing are commonly used stunning methods intended to immobilise pigs before exsanguination. Electrical stunning has been shown to cause a faster pH decline and inferior WHC than carbon dioxide stunning (Casteels *et al.*, 1995; Channon, Payne & Warner, 2000). This is due to the increase in post-mortem energy metabolism resulting from increased muscle activity post-mortem (Van der Wal, Engel & Hulsegge, 1997). Additionally, Van der Wal, Engel & Hulsegge (1997) found that 25% of all cases of aberrant pork quality had the electrical stunning procedure incorrectly administered which indicates that the correct administration of stunning can assist in the improvement of meat quality from electrically stunned pigs. PHu is not affected by stunning pigs using carbon dioxide (Casteels *et al.*, 1995; Channon *et al.*, 2003), nor does it often contribute to poor meat quality (Velarde *et al.*, 2000; Velarde *et al.*, 2001), and for this reason this technology is the best option for stunning pigs pre-slaughter.

The movement of pigs to the stunner has been indicated as a short-term pre-slaughter factor that can impact on meat quality. Grandin (1980) found that the more difficult it is to move the animal towards the stunner, the lower the WHC of the pork. This is supported by D’Souza *et al.* (1998) who determined that negative handling immediately pre-slaughter caused higher levels of surface exudate and higher incidence of PSE from pigs handled minimally pre-slaughter. This surface exudate, an indicator of a decreased WHC is most likely due to the
excitement induced by ushering, triggering lactic acid production and dropping the pH of the muscle causing protein denaturation and paler meat colour (Grandin, 1980).

### 1.5 Post-Slaughter Factors

#### 1.5.1 Chilling rate

The rate at which a carcass is chilled directly affects its meat quality due to the direct effects of temperature on post-mortem metabolism and its effect on the movement and distribution of water in muscle tissue (Rosenvold & Andersen, 2003). The exposure of carcasses to high temperatures at low pH is what leads to the denaturation of muscle proteins and the development of PSE-like characteristics (Hambrecht et al., 2004). Accelerated air chilling has been shown to improve WHC and reduce the incidence of PSE by rapidly dropping carcass temperature post-slaughter thereby reducing the effects of the post-mortem lactic acid accumulation on protein denaturation (Milligan et al., 1998; Springer et al., 2003). However, D’Souza et al. (1998) question the impact of accelerated chilling on meat quality due to its greater potential to cause cold shortening, which effectively offsets any positive effects of protein denaturation on pork tenderness. In addition, results obtained by Kerth et al. (2001) determined that accelerated chilling is effective at reducing the incidence of PSE in carriers of the halothane gene but does not reduce the incidence of PSE in non-carriers of the halothane gene.

Whilst accelerated or prompt chilling decreases the incidence of PSE, the opposite occurs when chilling rate is retarded. High body temperatures pre-slaughter or the delayed chilling of carcasses post-mortem causes rapid post-mortem glycolysis, accumulation of lactic acid and low pH tending towards the development of PSE pork (Tomović, Petrović, & Džinić, 2008). It is this combination of acidic conditions and high carcass temperature post-mortem that leads to protein denaturation and PSE formation (Honikel & Kim, 1986). Carcass temperature in relation to chilling rate, and post-mortem pH, are directly associated with technical pork quality attributes in this way.

#### 1.6 Conclusion

The biochemical mechanisms involved in the conversion of living pig muscle to pork are complex. Research has identified that the glycolytic potential, glycolytic rate and pH of pork
meat are the most significant contributors to the development of meat quality. This has numerous implications for pork quality improvement and subsequently, measurable technological meat quality attributes have been identified that are colour, water holding capacity, early post-mortem pH, pHu and glycolytic potential. However, a large number of animal attributes and pre- and post-slaughter factors impact on the metabolism of a pig before and after exsanguination making the implementation of strategies for meat quality improvement more difficult. Furthermore, continuous changes are made to production practices in order to fulfil economic and welfare objectives.

A feature of Australian pork production is towards semi-outdoor and outdoor housing systems such as eco-shelters and free-range. The pork quality implications of raising pigs in eco-shelters and extensive conditions in Western Australia remain largely unknown and the role these systems play on muscle metabolism under local conditions has not yet been identified. As discussed, the outcomes of previous meat quality studies into the effect of eco-shelters and free-range raising systems on meat quality attributes have been relatively inconclusive due to the high levels of variation in conditions between studies. Future studies relating directly to Western Australian pork production may yield results more applicable to the local industry.

It is proposed that pigs raised in free-range and eco-shelter conditions in Western Australia are more likely to have an increased incidence of darker and higher pHu pork carcasses than that of conventionally raised pigs. This is based on the above assessment of the literature, which determined that pigs housed in straw enriched environments have higher energy demands (Patton et al., 2008) causing a reduction in the glycolytic potential of pigs due to promoted foraging behaviour (Van de Weerd & Day, 2009). Factors that need to be taken into account in such a study are transportation, climate, genetics and differences in long-term and short-term stressors and slaughter plant conditions due to their contribution to the development of pork meat quality. A technological meat quality audit would therefore be the most appropriate study to determine the impact of free-range and eco-shelter conditions on pork meat quality in Western Australia.

Bibliography


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of outdoor rearing and indoor temperature on growth performance, carcass, adipose tissue and muscle traits in pigs, and on the technological and eating quality of dry-cured hams.


2.1 Abstract

An experiment was conducted to test the hypothesis that finishing pigs kept in single-group outdoor housing systems (eco-shelters) will have lower pre-slaughter muscle glycogen and 24 hour post-mortem lactate content, a higher 24 hour pH and darker meat colour than pigs finished in intensive shed systems. A total of 15 groups of pigs (n=704) sourced from two commercial finishing pig systems at Narrogin in WA were used in the study. Pigs of the same genotype and nutritional regime were kept in either intensive indoor shed systems or in eco-shelters, and then trucked to a commercial abattoir for slaughter. The groups were sampled between April and August 2012. Following slaughter, split carcasses were chilled and approximately 24 hours later, pH and colour was measured, samples of muscle from the longissimus dorsi taken and frozen for subsequent analysis of residual glycogen and lactate content. Eight groups of pigs had to be removed from the analyses due to unforeseen experimental complications, leaving a total of seven groups (n = 350) for final statistical analyses. In this regard it was not possible to statistically compare the effects of housing between kill days and the data demonstrated a significant difference in least-squares means between kill-groups on different kill days for all tested variables (P<0.05). Entire males had a lower 24 hour lactate level, lower a*, b*, HCW, P2 and higher 24 hour pH than females (P<0.05). Relationships between lactate and pH, pH and total glycogen, lactate and total glycogen, and lactate and residual glycogen were significant (P<0.05), with the latter two suggesting that pigs with more pre-slaughter glycogen have a faster rate of lactate accumulation post-mortem. Future research should be directed towards understanding the underlying mechanisms and under more controlled experimental conditions assess the influence of housing system before slaughter on pre-slaughter glycogen levels in order to manage pork pH.

2.2 Introduction

Variation in the pH of fresh pork following slaughter is a major cause of inconsistent pork meat quality (Scheffler & Gerrard, 2007), that in turn can be reflected by either pale soft and
exudative (PSE) meat or dark firm and dry (DFD) meat (Guàrdia et al., 2005; Shen et al., 2006). Known for its aberrant features, both PSE and DFD hinder meat processing and reduce market appeal, and in the USA pork industry, has been reported as costing in excess of $100 million per annum (Shen et al., 2006; Scheffler & Gerrard, 2007).

The level of stored muscle glycogen at slaughter generally determines the extent of post-mortem glycolysis, thus having a major impact on meat quality. Glycogen in muscle post-mortem undergoes anaerobic metabolism via glycogenolysis, glycolysis and lactic acid fermentation (Shen et al., 2006; Choe et al., 2008). Different levels of accumulated lactate and hydrogen ions from the conversion of glycogen to lactic acid is one of the main causes of variation in meat quality attributes due to its affect on muscle pH (Ryu & Kim, 2006). High levels of muscle glycogen pre-slaughter increase the potential lactic acid production post-mortem, lowering the pH of the meat and increasing the risk of PSE pork (Scheffler & Gerrard, 2007). In contrast, pigs having very low levels of muscle glycogen pre-slaughter have a limited capacity to produce lactic acid post-mortem resulting in meat with a higher pH, thereby increasing the risk of DFD pork (Guàrdia et al., 2005). This suggests that any factors that might impact on the amount of glycogen stored in muscle before slaughter could influence pork pH, and consequently its quality and attributes.

Particular factors that influence glycogen stores and the rate and extent of post-mortem glycolysis include genetics, feed intake, animal handling, post-slaughter chilling rate and (or) the type of production system (Van der Wal, Engel & Hulsegge, 1997; Terlouw, Berne & Astruc, 2009). Of these factors and peculiar to the Australian pig production system, the impacts of the housing system of finishing pigs (i.e., those destined for market) have received scant attention. Finishing pigs under Australian conditions are grown in either intensive indoor shed systems, where pigs are housed in groups on concrete flooring, or in single-group outdoor housing systems called hoops or eco-shelters. Pigs finished in eco-shelters show increased levels of physical activity, foraging behaviour and pig-to-pig interaction compared to pigs kept in enclosed indoor systems (van de Weerd & Day, 2009). This increase in physical activity is likely associated with depletion of muscle glycogen stores before slaughter, suggesting a possible impact on pork pH and other meat characteristics such as colour and water-holding capacity (Patton et al., 2008).
In this experiment, I hypothesised that finishing pigs kept in eco-shelters and then slaughtered at a commercial abattoir will have lower pre-slaughter muscle glycogen content, a lower 24 hour lactate content and as a result, a higher 24 hour pH and darker meat colour than pigs finished in intensive shed systems. The overall aim of the study was to assess the impact of these two different housing systems on aspects of pork meat related to pH change.

2.3 Materials and Methods

2.3.1 Animals, housing and feed

The study was carried out between April and August 2012, with permission obtained for the removal of dead animal tissue from third party sources at the Linley Valley Fresh abattoir, Wooroloo, approved by the Murdoch University Animal Ethics Committee.

A total of 704 pigs of Large White x Landrace x Duroc origin (Myora Pig Genetics, South Australia) were allocated to the study. Pigs were derived after weaning from the free-range breeding facility of Craig Mostyn Farms, Albany, which were transported to the Craig Mostyn Farms grow out operation, at Narrogin, until a slaughter weight of approximately 90 kg was reached. Separate housing systems at the Narrogin facility allowed two different housing treatments to be investigated. Intensive shed housing was one housing treatment and comprised conventional grower-finisher sheds with concrete slatted floors and steel tubular fencing. Pigs were penned in groups of 12 with no additional bedding. The second housing treatment was in eco-shelters consisting of a concrete base and plastic or canvas roof stretched over an arched metal frame. Each eco-shelter had straw bedding and contained 140 pigs. Pigs in each housing type were not moved between housing treatments after initial placement at the Narrogin facility. The pigs in the shed system were supplied a diet in meal form from metal troughs whilst pigs in the eco-shelters were provided a diet in pelleted form from barrel feeders. Pigs from both housing treatments were fed ad libitum exactly the same series of commercially formulated diets (supplied by Wesfeeds, Welshpool, WA) over the entire growth period. Pigs were also offered water ad libitum via nipple drinkers. Craig Mostyn staff and associated contractors undertook all animal husbandry, transport and slaughter in accordance with standard facility procedures and protocols.
2.3.2 Transport, lairage and slaughter

Pigs were transported by truck once an on-farm target slaughter weight of approximately 90 kg was reached (Gyula Hegedus, Farm Manager at Craig Mostyn Farms Narrogin; personal communication). Pigs were to be transported in groups of 100 or more, with each consignment carrying both shed-finished and eco-shelter-finished pigs. Pigs were ushered onto trucks using best practice procedures by Narrogin piggery staff with pigs from different housing systems separated by partition on-board the vehicle. The same driver and trailer design were used to transport the pigs across the entire sampling period. Upon arrival at Linley Valley Fresh Pork abattoir (Wooroloo, WA), pigs were ushered off the truck into a lairage facility with each housing group placed into separate pens. Pigs were then ushered to the stunner by trained livestock personnel and abattoir slaughter procedures were followed. This included stunning by CO$_2$ gas prior to exsanguination and processing. The hot carcasses were split in half and hung by the Achilles tendon for chilling; hot carcass weight and back fat depth were measured at the P2 site, located 6.5 cm from the midline over the last rib, prior to being transported to the chiller. Time in lairage was calculated as time from arrival at the abattoir until scanned after slaughter.

2.3.3 Experimental Design

Pork samples, pH, temperature and colour measurements were to be taken from 30 randomly selected shed-finished pigs (15 male, 15 female) and 30 randomly selected shelter-finished pigs (15 male, 15 female) transported and slaughtered at the same time on the same day each week for 15 weeks spanning April to August. Samples were to be taken at 0730 the day after slaughter and placed on ice until transferred to the laboratory where samples were frozen at -20° C until chemical analysis.

2.3.4 Sample Collection

2.3.4.1 pH, Carcass Temperature, Colour and Meat Sampling

The pH was measured using a pH 300 hand-held pH/mV/temperature meter (Eutech instruments, Singapore) fitted with a temperature and IJ44C intermediate junction pH probe (Ionode, Tennyson). The pH meter was calibrated on two standards (pH 4.01 and 7.0) as per manufacturers instructions. The probe was inserted into the m. longissimus dorsi (LD) of each
carcass from between the 3rd and 4th ribs 7.5 cm from the ventral edge of the split pork carcass and temperature was taken from the same location. Muscle samples were taken using a metal meat punch between the ribs at the same location as the pH probe was inserted. This allowed for the removal of approximately 1 g muscle tissue from the LD muscle without adversely affecting the subsequent processing of the carcass. Fat and residuals of other muscles were removed and samples were kept on ice until frozen at the laboratory.

Colour measurements were taken using a Chroma meter CR-400 (Minolta, Osaka, Japan). The Chroma meter was calibrated as per manufacturers instructions by reading a white tile multiple times. Samples were measured by pressing the measuring head to the uncut surface of the exposed TA muscle inside each carcass. The muscle surface was measured using the CIE L*, a*, b* system using a measuring head standardised to a white tile, with D65 lighting, a 2 standard observer and 8mm aperture. Measurements for L* represent lightness, whilst a* represents a relative redness/greenness and b* a relative yellowness/blueness.

2.3.5 Chemical analysis

2.3.5.1 Lactate and glycogen assays

Analysis of the muscle samples collected were chemically analysed for lactate and residual glycogen content at Murdoch University (Perth, Western Australia). The glycogen assay was based on the enzymatic method of Chan & Exton (1976) but excluding the filter paper step, and the lactate assays used the same homogenate. Briefly, 250mg of frozen LD muscle samples were weighed out into test tubes and kept on ice. Next, 2.5 mL of 30 mM hydrochloric acid was added to the test tubes and the sample was homogenized for 30 seconds using a Polytron (Bosch GGS 27C Professional) and left to settle whilst on ice for 1 – 2 hours. Sample liquid (not foam), was transferred to Eppendorf tubes and frozen at -20 °C until required. The auto analyser (Olympus AU 400, Olympus Diagnostics, Tokyo, Japan) used for completing the analysis was calibrated using lactate and glucose standards according to the machine manual, then 60 µl of defrosted, vortex spun samples were pipetted into auto analyser cups and analysed for lactate. Glycogen in each of the remaining homogenate samples was then broken into glucose for analysis in the auto analyser, by combining 125 µl of homogenate with 125 µl of distilled water and 1 mL of an enzyme and acetate buffer mixture (0.0128 mg amylase, 0.0128 mg amyloglucosidase in 80 mL of pH 4.8, 40 mM acetate buffer (0.41 g sodium acetate, 0.3g glacial acetic acid and distilled water)) and incubating in shaking water bath at 37 °C for 60 minutes. This mixture was then pipetted in
60 µl aliquots into auto analyser cups and analysed for glucose content to establish the amount of total residual glycogen in the muscle sample (number of moles of glucose reflects the number of moles of glycogen in the sample). Total glycogen was calculated by adding the number of moles of residual glycogen with half the number of moles of lactate (equivalent of two lactate molecules to every one glucose or glycogen) and converting to g per 100g of muscle sample.

2.3.6 Statistical analysis
The software package SAS® was used for all statistical analyses (SAS Institute, 2001). Lactate, residual glycogen, total glycogen, L*, a*, b* and pH data was analysed using a linear mixed effects model using sex and housing type within kill-group as fixed effects. A kill-group represented the day that pigs were killed on, thus each housing term was analysed within this effect. A separate analysis was carried out and analysed the model for pH with the inclusion of Lactate and glycogen as separate covariates. Glycogen was also analysed the same with the inclusion of lactate as a covariate, and lactate analysed with residual glycogen as a covariate. This enabled the biological interaction of these terms to be investigated. Outliers resulting from the lactate and glycogen analysis were removed due to values that were considered too high and were likely due to human error. Statistical significance was accepted at P < 0.05.

2.4 Results

2.4.1 Data removal and complications
Due to unforeseen and uncontrollable circumstances associated with chiller malfunction, insufficient identification of the correct housing treatment on-farm and pig identification issues (see Discussion for greater detail of these issues), of the 704 pigs sampled, only a total of 350 pigs could be statistically analysed in the study. The remainder were removed and not considered any further. Data are therefore presented as least-squares (LS) means representing the seven kill-groups for which pigs from one of the two housing systems were sampled on each day.

2.4.2 pH, Lactate and glycogen
The statistical outputs for pH, lactate and glycogen contents are presented in Table 1. There was an effect of kill-group for pH, lactate, residual glycogen and total glycogen (P<0.05; Table 1). Kill-group 7 (April) had the lowest pH and the highest lactate, whilst kill-group 14
Table 1: The statistical outputs for the linear mixed effect analysis for all continuous variables

Table 2: Least squared means and associated standard error (SE) of pH, lactate, total glycogen and residual glycogen for each kill group.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Kill Group (Housing)</th>
<th>N</th>
<th>Sex</th>
<th>Sex x Kill Group (Housing)</th>
<th>NDF;DDF</th>
<th>F Value</th>
<th>P Value</th>
<th>NDF;DDF</th>
<th>F Value</th>
<th>P Value</th>
<th>NDF;DDF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate (umol/g)</td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Glycogen (g/100g)</td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual Glycogen (umol/g)</td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat depth (cm)</td>
<td></td>
<td>39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means in a column having the same superscript do not differ; statistical difference is determined at p < 0.05.
Table 3: Least squared means and associated standard error (SE) of the sex interaction for all terms

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>SE</th>
<th>Female</th>
<th>SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>5.78</td>
<td>0.0147</td>
<td>5.74</td>
<td>0.0148</td>
<td>0.038</td>
</tr>
<tr>
<td>Lactate</td>
<td>98.55</td>
<td>0.9096</td>
<td>101.45</td>
<td>0.9167</td>
<td>0.024</td>
</tr>
<tr>
<td>Total Glycogen</td>
<td>1.03</td>
<td>0.0148</td>
<td>1.07</td>
<td>0.0150</td>
<td>0.08</td>
</tr>
<tr>
<td>Residual Glycogen</td>
<td>8.20</td>
<td>0.5397</td>
<td>8.76</td>
<td>0.544</td>
<td>0.461</td>
</tr>
<tr>
<td>$L^*$</td>
<td>37.72</td>
<td>0.1978</td>
<td>38.21</td>
<td>0.1994</td>
<td>0.081</td>
</tr>
<tr>
<td>$a^*$</td>
<td>15.10</td>
<td>0.1455</td>
<td>16.25</td>
<td>0.1467</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>$b^*$</td>
<td>7.76</td>
<td>0.1312</td>
<td>8.32</td>
<td>0.1323</td>
<td>0.003</td>
</tr>
<tr>
<td>HCW</td>
<td>68.24</td>
<td>0.437</td>
<td>69.78</td>
<td>0.4404</td>
<td>0.013</td>
</tr>
<tr>
<td>P2 Fat (mm)</td>
<td>9.11</td>
<td>0.1648</td>
<td>9.86</td>
<td>0.1661</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 4: Least squared means and associated standard error (SE) of colour measurements $L^*$, $a^*$ and hot carcass weight and P2 fat depth for each kill group (housing)

<table>
<thead>
<tr>
<th>Kill Group</th>
<th>Housing</th>
<th>N</th>
<th>Estimate</th>
<th>SE</th>
<th>Estimate</th>
<th>SE</th>
<th>Estimate</th>
<th>SE</th>
<th>Estimate</th>
<th>SE</th>
<th>Estimate</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Shed</td>
<td>39</td>
<td>37.32</td>
<td>0.417</td>
<td>14.04</td>
<td>0.307</td>
<td>9.54</td>
<td>0.347</td>
<td>7.01</td>
<td>0.276</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Shed</td>
<td>40</td>
<td>37.61</td>
<td>0.411</td>
<td>16.73</td>
<td>0.303</td>
<td>9.40</td>
<td>0.343</td>
<td>8.30</td>
<td>0.273</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Eco</td>
<td>40</td>
<td>37.84</td>
<td>0.411</td>
<td>14.88</td>
<td>0.303</td>
<td>10.45</td>
<td>0.343</td>
<td>7.59</td>
<td>0.273</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Eco</td>
<td>61</td>
<td>40.07</td>
<td>0.333</td>
<td>15.98</td>
<td>0.245</td>
<td>9.96</td>
<td>0.277</td>
<td>8.79</td>
<td>0.221</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Shed</td>
<td>53</td>
<td>7.39</td>
<td>0.395</td>
<td>16.67</td>
<td>0.339</td>
<td>9.45</td>
<td>0.345</td>
<td>8.25</td>
<td>0.283</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Eco</td>
<td>59</td>
<td>37.67</td>
<td>0.339</td>
<td>15.46</td>
<td>0.249</td>
<td>8.99</td>
<td>0.282</td>
<td>8.14</td>
<td>0.251</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Eco</td>
<td>58</td>
<td>38.16</td>
<td>0.342</td>
<td>15.88</td>
<td>0.251</td>
<td>8.14</td>
<td>0.285</td>
<td>8.31</td>
<td>0.251</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Eco</td>
<td>58</td>
<td>38.16</td>
<td>0.342</td>
<td>15.88</td>
<td>0.251</td>
<td>8.14</td>
<td>0.285</td>
<td>8.31</td>
<td>0.251</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: The symbol * indicates significance at the 5% level.
(August) had the highest pH and the lowest lactate (P<0.05; Table 2). This resulted in a difference between means that was in the order of 0.22 pH units and 10.7 µmol/g of lactate (P<0.05). For total and residual glycogen, kill-group 13 (July) had the highest concentration of both, while kill-group 11 (July) had the lowest (P<0.05; Table 2). From kill-group 11 (July) to kill-group 13 (July) residual glycogen at 24 hours doubled while total glycogen varied by 0.13 g/100g (Table 2).

There was also an effect of sex on pH and lactate only (Table 1), with males producing 2.9 µmol/g less lactate (P<0.05) and having a having a pH 0.04 units higher (P <0.05) than females (Table 3).

### 2.4.3 Colour

The statistical outputs for effects on the colour of the meat are presented in Table 1. There was a significant effect of kill-group for L*, a* and b* (P < 0.05; Table 1) and an effect of sex for a* and b* (P < 0.05; Table 1) only. Kill-group 11 (July) had the lightest *m. transversus abdominus* (TA) with the highest L* value, which was 2.96 units higher than kill-group 12 (July), which had the darkest muscle (lowest L* value) (Table 4). The reddest TA muscles, measured by a*, were found in kill-group 12 (July), which was 2.74 units higher than the least red muscle in kill-group 1 (April) (Table 4). The blueness of the TA muscle, measured by b*, was highest in kill-group 9 (May) and lowest in kill-group 14 (August) with a difference between them of 2.31 units (Table 4).

The sex effect identified for a* demonstrated that females had a redder TA muscle by 1.15 units than males (P<0.05; Table 1). Similarly, females had a 0.56 units higher b* value for blueness than males (P<0.05; Table 3).

### 2.4.1 Carcass characteristics

The statistical outputs for the analysis of hot carcass weight (HCW) and backfat depth (P2) are shown in Table 1. Although significant kill-group(housing) and sex effects existed (Table 3) for P2, the P2 was influenced by the significant interaction (P<0.05; Table 1) between killgroup(housing) and sex that was driven by the difference between males (8.3 ± 0.46 mm) and females (12.6 ± 0.46 mm) from kill-group 9 (data not shown).

There was also a significant kill-group effect and sex effect for HCW (P < 0.05; Table 1). Male carcasses were 1.54 kg lighter and had 0.75 mm less back fat than females (Table 3).
The HCW and P2 also varied between kill-groups (P<0.05; Table 1 and 4). The lowest mean HCW coincided with the highest P2 fat depth (kill-group 11, July), while no other trends were observed. The range in HCW extended over 8.3 kg, whilst the range for P2 fat extended over 1.8 mm (Table 4).

### 2.4.2 Lactate, glycogen and pH interactions

The pH of the LD muscle at 24 hours (pH 24) post-mortem decreased, with more lactate being present at 24 hours post-mortem. This is represented by the curvilinear relationship in Figure 1 and the statistical output for this analysis is shown in Table 5. A similar curvilinear relationship was observed between pH 24 and total glycogen at slaughter (Figure 2; see Table 6 for statistical output) with an increase in glycogen stores at slaughter resulting in a lower pH at 24 hours post-mortem. However, at glycogen levels greater than about 1.1 g/100g of tissue, the effect of this relationship began to plateau (Figure 2). Generally, an increase in glycogen at slaughter from 0.6 to 1.2 g/100g resulted in a decrease of approximately 0.5 of a pH unit at 24 hours.

Lactate content in the muscle at 24 hours post-mortem and total glycogen at slaughter showed a significant linear relationship (P<0.05; Table 7), with more total glycogen at slaughter producing more lactate in the muscle 24 hours post-slaughter (Figure 3). Additionally, a curvilinear relationship between lactate and residual glycogen, both at 24 hours post-mortem, was present (Figure 4; see Table 8 for statistical outputs). Therefore, when lactate levels are high at 24 hours post-mortem, it is more likely that residual glycogen pools will also be high at the same time point. Furthermore, if levels of lactate were as low as about 90µmol/g it was likely that after 24 hours no residual glycogen would remain.
Table 5: Statistical model output for pH interacted with lactate, 24 hours post mortem

<table>
<thead>
<tr>
<th>Term</th>
<th>NDF;DDF</th>
<th>F Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>1;321</td>
<td>0.13</td>
<td>0.7166</td>
</tr>
<tr>
<td>lactate</td>
<td>1;321</td>
<td>27.59</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>housing(killgroup)</td>
<td>6;321</td>
<td>0.77</td>
<td>0.5945</td>
</tr>
<tr>
<td>lactate x housing(killgroup)</td>
<td>6;321</td>
<td>0.93</td>
<td>0.4734</td>
</tr>
<tr>
<td>housing x sex(killgroup)</td>
<td>6;321</td>
<td>3.2</td>
<td>0.0046</td>
</tr>
<tr>
<td>lactate x sex</td>
<td>1;321</td>
<td>0.18</td>
<td>0.6735</td>
</tr>
<tr>
<td>lactate x housing x sex(killgroup)</td>
<td>6;321</td>
<td>2.88</td>
<td>0.0096</td>
</tr>
<tr>
<td>lactate x lactate</td>
<td>1;321</td>
<td>16.8</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Figure 1: The relationship between pH and lactate at 24 hours post mortem for all pigs. Data points are the residuals of the least squared means.
Table 6: Statistical model output for pH at 24 hours post-slaughter interacted with total glycogen content pre-slaughter.

<table>
<thead>
<tr>
<th>Term</th>
<th>NDF;DDF</th>
<th>F value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>1;321</td>
<td>0</td>
<td>0.992</td>
</tr>
<tr>
<td>glycogen</td>
<td>1;321</td>
<td>108.44</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>housing(killgroup)</td>
<td>6;321</td>
<td>2.21</td>
<td>0.0423</td>
</tr>
<tr>
<td>glycogen x glycogen</td>
<td>1;321</td>
<td>60.49</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>sex x housing(killgroup)</td>
<td>6;321</td>
<td>3.42</td>
<td>0.0028</td>
</tr>
<tr>
<td>glycogen x sex</td>
<td>1;321</td>
<td>0</td>
<td>0.9533</td>
</tr>
<tr>
<td>glycogen x housing(killgroup)</td>
<td>6;321</td>
<td>2.03</td>
<td>0.0611</td>
</tr>
</tbody>
</table>

Figure 2: The relationship between total glycogen and pH at 24 hours post mortem for all pigs. Data points are the residuals of the least squared means.
Table 7: Statistical model output for lactate at slaughter interacted with total glycogen at 24 hours post mortem

<table>
<thead>
<tr>
<th>Term</th>
<th>NDF;DDF</th>
<th>F Value</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>sex</td>
<td>1;327</td>
<td>6.15</td>
<td>0.0136</td>
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<tr>
<td>glycogen</td>
<td>1;327</td>
<td>260.47</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>housing(killgroup)</td>
<td>6;327</td>
<td>4.02</td>
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</tr>
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<td>glycogen x housing (killgroup)</td>
<td>6;327</td>
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<td>0.0008</td>
</tr>
<tr>
<td>glycogen x sex</td>
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</tr>
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<td>glycogen x glycogen x housing (killgroup)</td>
<td>6;327</td>
<td>3.85</td>
<td>0.001</td>
</tr>
<tr>
<td>glycogen x glycogen</td>
<td>1;327</td>
<td>144.54</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Figure 3: The relationship between total glycogen and lactate at 24 hours post mortem for all pigs. Data points are the residuals of the least squared means
Table 8: Statistical model output for lactate interacted with residual glycogen, 24 hours post mortem

<table>
<thead>
<tr>
<th>Effect</th>
<th>NDF:DDF</th>
<th>F Value</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>residual glycogen</td>
<td>1:341</td>
<td>100.15</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>housing(killgroup)</td>
<td>6:341</td>
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</tr>
<tr>
<td>residual glycogen x residual glycogen</td>
<td>1:341</td>
<td>51.09</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

2.5 Discussion

2.5.1 Effects of housing on meat characteristics - pH, lactate, glycogen and colour

The overall aim of this study was to determine the effect of different finishing-housing systems on the pH-related meat characteristics of pork. I examined the hypothesis that pigs finished in eco-shelters would have a lower total muscle glycogen before slaughter, lower lactate accumulation, higher pH in the LD muscle and a darker meat colour in the TA muscle at 24 hours post-mortem compared to pigs finished in a conventional indoor (shed) finishing system. Preliminary statistical analyses showed there was a significant effect of housing across all measures, however this effect was confounded within the corresponding kill-group, i.e., the day pigs were killed. Therefore, acceptance of the current hypothesis remains
inconclusive. The effect of kill-group was essentially an effect which may be associated with environmental, farm and (or) abattoir factors, and these factors are known to influence variation in pork meat quality (Terlouw et al., 2008). Controlling and accounting for the variation of factors such as weather, handling and pig activity between the day pigs were slaughtered is difficult in applied industry research, thus the statistical analysis was required to adjust for these differences by the inclusion of kill-group as a variable.

In this regard, a major downfall of industry-applied research is that sometimes the planned experimental designs can be difficult to execute. The current study set out to sample carcasses of both sexes from both housing treatments on the same day (i.e., the same kill-group). Unfortunately, due to unforeseen and uncontrollable circumstances, the current study was not able to deliver on the proposed experimental design. Of the 704 total pigs sampled over 15 separate days, only 350 pigs sampled over 7 days were able to be included in the statistical analyses. Samples collected on 3 of the 15 sample days, mixed shed and shelter treatments together during transport, thus there was no way of distinguishing what housing treatment each carcass came from, thus were removed from the analyses. On a further five sampling days, complications arose in the abattoir associated with a malfunctioning chiller having a temperature greater than 4 °C. A retarded chilling rate is known to enhance post-mortem glycolysis as muscle enzymes maintain a higher level of activity in warmer temperatures (Rosenvold & Andersen, 2003). Thus, these samples were removed.

The male pigs in the study had a higher pH 24 with a corresponding lower lactate level than female pigs (P<0.05; Table 3). This finding is in agreement with work by Van der Wal, Engel, & Hulsegge (1997) whom reported that male pigs had a higher pHu and less surface exudate than female pigs. This could be attributed to the higher level of fighting and aggression that occurs between male pigs in the time before slaughter (Guàrdia et al., 2005). Fighting behaviours are associated with a higher incidence of DFD due to the expenditure of muscle glycogen pre-slaughter (Guàrdia et al., 2005). Conversely, other studies reported minor effects of gender on meat quality (Jeremiah et al., 1999).

Colour measurements for lightness (L*) do not conform to published work that reported male pigs as having darker meat (Channon, Kerr & Walker, 2004). In the current study there was no sex effect for L*. Huff-Lonergan et al. (2002) determined that L* correlated with pHu and that the higher the pHu the darker the meat colour. This relationship was also observed in the
current study for pH and L* at 24 hours post-slaughter (P<0.05; results not shown). Free-range finished pigs have been demonstrated by Lambooij et al. (2004) to have a higher pHu than non-free-range finishing pigs. Although no comparison can be made between housing conditions in the current study, a change in housing conditions, shifting towards a more free-range system, may be a way of managing pH and meat colour for controlling meat quality.

The significantly lower HCW (P<0.05) and P2 values (P<0.05) in male pigs are supported by the work of Babol & Squires (1995), which found that male pigs were generally leaner than female pigs. At the same stage of maturity, entire male pigs are generally heavier than female pigs (Dunshea et al., 2003), however data from the current study showed the opposite. It is likely that there may have been some selection of carcasses for sampling at the abattoir that distorted the real differences in HCW between males and females. Female pigs are preferred over male pigs for export because of a greater product consistency due to no boar taint (Bonneau et al., 2000), and abattoir personnel may have deliberately excluded many of the lean lower weighted females from the sampling group to use for the export market. This may have negatively impacted on sex differences causing inflated or deflated statistical differences between males and females.

### 2.5.2 Influence of pre-slaughter muscle glycogen levels on lactate and pH

Lactate and pH at 24 hours post-slaughter were associated as anticipated; as the lactate content increased, the pH decreased (Figure 1; Table 5). This relationship can be explained simply by post-mortem glycolysis, that is, as lactate was produced anaerobically post-mortem, the pH of the muscle tissue declined in response to increasing lactic acid levels (Scheffler & Gerrard, 2007). This relationship confirms that glycolysis and lactic acid production occurred and is also reflected in the differences of the kill-group analyses, where kill-group 14 (Eco-shelter, August) had the highest pH and the lowest lactate, and kill-group 7 (Shed, April) had the highest pH and the lowest lactate.

The interaction between total glycogen at slaughter and pH 24, demonstrated a relationship that suggests that a higher glycogen store at slaughter promoted as faster the rate of post mortem glycolysis as indicated by a lower at pH at 24 hours (Figure 2; Table 6). Furthermore, the linear relationship between total glycogen at slaughter and lactate production at 24 hours post-slaughter (Figure 3; Table 7) is also evident of an increased rate of post mortem
glycolysis associated with larger glycogen pools. However, lactate is a component of the calculation of total glycogen, meaning this relationship is biased. Moreover, the association between lactate production and residual glycogen at 24 hours post slaughter (Figure 4; Table 8) is a positive indicator that larger glycogen pools are increasing the lactate production at 24 hours post mortem. When a large amount of lactate had been produced at 24 hours post-slaughter, as associated with an increased rate of glycolysis, the more likely a larger residual of glycogen would be present. This work is supported by Choe et al. (2008) who found that muscles with low glycogen and lactate levels demonstrated normal rates of post-mortem glycolysis, whilst muscles that contained high glycogen and lactate levels had a more rapid post-mortem glycolytic rate.

This finding assumes, however, that none of the samples had reached pHu. The pH of meat is considered to reach pHu when glycolysis has ceased and thus no further production of lactate or lowering of pH occurs. If this is assumed then this rate-like relationship could be explained by the Michaelis-Menten theory of enzyme kinetics. This theory states that the more substrate that is available for an enzyme to utilise, then the faster the production of product will be until maximum velocity is reached and enzymes have reached saturation (Lehninger et al., 2005). It is unlikely in this circumstance that none of the samples reached pHu, particularly given that a number of samples that contained no residual glycogen at 24 hours post-mortem. So whilst, the association of lactate and total glycogen, and pH and total glycogen may represent a rate of lactate accumulation or pH decline, it is impossible to determine from the data collected in this experiment if a sample had reached pHu. None-the-less, if a greater concentration of glycogen does in fact increase the rate and extent of post mortem glycolysis, this may be implicated into the management strategies of the pork industry to monitor meat quality.

Currently in Australia, circumstantial evidence suggests that the pHu of pork is declining over time. Given that consumers prefer pork at a higher pHu (Bryhni et al., 2003), it would be beneficial to determine methods for which this national pH decline can be managed. The relationships described here suggest that the more glycogen present in pig muscle pre-slaughter, the faster the rate of pH decline and therefore the greater the potential to reach a low pH within 24 hours of slaughter. While no conclusions could be drawn as to their efficacy in managing glycogen stores, finishing-housing systems may still be a potential method for the management of glycogen levels pre-slaughter. The current understanding of pre- and post-
mortem metabolism provides enough background to further investigate the effect of housing and the effect on glycogen pre-slaughter. These future studies must be aware of the complications of the possible confounding effects of kill groups and should be strictly controlled. The manipulation and monitoring of glycogen pre-slaughter may be required to manage pork pH and optimize quality characteristics in the future. However, a more comprehensive understanding of the relationships between finishing systems, glycogen and pH is required as identified in the current study.

2.5.3 Conclusion
In conclusion, due to complications involved in sample collection, no effect of housing on the pork quality characteristics of pH, glycogen, lactate or colour could be established due to the confounding effects of kill day. However, there is a difference in certain meat quality attributes due to sex, as males have a higher pH 24 and lower lactate level 24 hours post-slaughter than female pigs (P<0.05). Additionally, significant correlations exist between pH and lactate (P<0.05; Figure 1; Table 5), total glycogen and pH (P<0.05; Figure 2; Table 6), total glycogen and lactate (P<0.05; Figure 3; Table 7) and residual glycogen and lactate (P<0.05; Figure 4; Table 8). As described above these findings conform to current understandings of pork quality and also develop further, the ideas implicating muscle glycogen stores as a contributor in determining the rate of post-mortem glycolysis.

2.6 References


