Effect of Laying Hen Housing Environment and Genetic Strain on Meat Quality and Skeletal Muscle Physicochemical Properties

by

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Abstract:

Skeletal muscle is an adaptable, multi-faceted tissue that is essential for whole body movement and metabolism. When a production animal is slaughtered, its muscle goes through complex physical and biochemical changes including a shift from aerobic to anaerobic metabolism resulting in the breakdown of glycogen. Glycogen breakdown and consequent lactate accumulation lead to a decline in muscle pH which influences meat quality traits such as colour and tenderness. Ante-mortem conditions such as physical activity, physiological stress and an animal's genotype can all affect meat quality. In conventional laying hen cages (CC), lack of physical space and inability to perform highly motivated behaviours leads to stress and inactivity. Due to the concern for hen welfare in this system, CC have been banned or are being phased out in various parts of the world. Furnished cages (FC) are an alternative to CC as they permit the expression of highly motivated behaviours and load-bearing activity which has been shown to improve hen humeral bone strength and reduce aggressive behavioural expression as compared to hens housed in CC. However, FC typically house larger group sizes than CC, thereby contributing to social stress.

In the first study presented in this thesis, the objective was to evaluate the effects of CC and FC laying hen housing environments and strain differences on meat quality of 80 to 81 week old birds. *Pectoralis major* meat quality was assessed for two flocks of Shaver White (SH), Lohmann Lite (LL) and Lohmann Brown (LB) hens housed in either 5-hen CC or 40-hen FC. Between 80 and 81 weeks, muscle samples were collected from randomly selected hens and analyzed for muscle pH, colour and shear force (SF) using established methods. In both flocks, the combined treatment body weights (BW) were higher for CC than FC hens and the combined strain BWs were higher for LB than LL and SH hens. Flock 1 LB had lower initial and ultimate pH than SH and LL, and greater pH decline than SH. Muscle redness (a*) was higher for CC SH

than FC SH in both flocks. Muscle a* was higher for LL than SH and LB in flock 1, and higher than SH in flock 2. Housing differences in muscle SF were absent. In CC, SF was higher for SH than LL and LB in flock 1, and higher than LB in flock 2. Lack of housing differences suggest that environmental stressors present in both housing systems similarly affected meat quality. Strain differences for muscle pH, a* and SF suggest increased stress experienced by SH and LL hens. The absence of flock 2 strain differences are consistent with a cannibalism outbreak that occurred in this flock and most severely impacted LB hens.

Post-mortem muscle pH decline has traditionally been attributed to glycogenolysis-induced lactate accumulation. However, muscle pH ($[H^+]$) is controlled by complex physicochemical relationships encapsulated in the Stewart Model of acid-base chemistry (Can. J. Physiol. Pharm. 61: 1444-61, 1983), and is determined by three systems-independent variables – *strong ion difference([SID]), total concentration of weak acids($[A_{tot}]$) and partial pressure of CO_2 (PCO_2)*. A second study therefore investigated the three systems-independent variables within *Pectoralis major* muscles of flock 1 hens, and evaluated the Model by comparing measured $[H^+]$ with calculated $[H^+]$. The Model proved exceptional, accounting for 99.7% of the variation in measured muscle $[H^+]$. Differences in [SID] accounted for most or all of the variation in $[H^+]$ between strains. Greater PCO_2–induced $[H^+]$ in FC compared with CC was counteracted by greater sequestration of strong base cations. The results demonstrate the accuracy and utility of the Stewart Model for investigating determinants of meat $[H^+]$. Additionally, the housing differences identified in this study suggested that hens housed in FC have improved muscle function and overall health due to the increased opportunity for movement. These findings, which were not apparent from the traditional meat quality measures conducted, support past studies showing improved animal welfare for hens housed in FC

compared to CC. Therefore, the Stewart model has been identified as an exceptional method to assess changes in the muscle at a cellular level that affect meat quality and reveal differences in the welfare status of the research subjects.

Preface:

Some of the research conducted for this thesis forms part of a research collaboration, led by Dr. M. J. Jendral at Dalhousie Agricultural Campus, formerly the Nova Scotia Agricultural College. This research project received ethics approval from Animal Care and Use Committee, Nova Scotia Agricultural College.

This thesis also contains research conducted at University of Alberta which received ethics approval from the Animal Care and Use Committee for Livestock, University of Alberta.

Chapter 2 has been submitted and accepted as K.M. Frizzell, E. Lynch, B.M. Rathgeber, W.T. Dixon, C.T. Putman and M.J. Jendral. Effect of housing environment on laying hen meat quality: Assessing *Pectoralis major* pH, colour and tenderness in three strains of 80-81 week old layers housed in conventional and furnished cages. *British Poultry Science*, 2016. My contributions to this article included data collection, data analysis and interpretation, and manuscript composition. E. Lynch and B.M. Rathgeber were involved with data collection and manuscript edits. W.T. Dixon and C.T. Putman were involved with manuscript edits. M.J. Jendral was the supervisory author for this research and was involved with concept formation, data collection, data interpretation, and manuscript composition and edits.

Chapter 3 has been submitted as K.M. Frizzell, M.J. Jendral, I.M. MacLean, W.T. Dixon, and C.T. Putman. Physicochemical determinants of pH in *pectoralis major* of three strains of laying hens housed in conventional and furnished cages. *Meat Science*, 2016. My contributions to this

article included method development, data collection, data analysis and interpretation, and manuscript composition. M.J. Jendral was involved with concept formation, method development, data collection and interpretation, and manuscript composition and edits. I.M. MacLean was involved with method development, data collection and manuscript edits. W.T. Dixon was involved with concept formation and manuscript edits. C.T. Putman was the supervisory author for this research and was involved with concept formation, method development, data collection, data analysis and interpretation and manuscript composition and edits.

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List of Abbreviations:

Housing Systems:

CC, conventional cage

FC, furnished cage

Genetic Strains:

SH, Shaver White

LL, Lohmann Lite

LB, Lohmann Brown

Variables Measured:

BW, hen body weight between 80 and 81 weeks

BD, breast muscle depth

pH_{17}, initial pH at 17 min post-mortem

pH_u, ultimate pH at 24 h post-mortem

ΔpH, pH decline between 17 min and 24 h post-mortem

L*, muscle lightness

a*, muscle redness

b*, muscle yellowness

SF, muscle shear force

BBS, bone breaking strength

TTW, total tissue water

SID, strong ion difference

$[A_{tot}]$, total concentration of un-dissociated weak acids

$[A^-]$, anion gap

P_{CO_2}, partial pressure of carbon dioxide

CHAPTER 1: Introduction and Literature Review

1 Introduction:

Skeletal muscle is a complex, adaptable organ that plays a major role in whole body structure and metabolism (Valberg, 2008). In animal production, once an animal has been slaughtered, muscle goes through many complex physical and biochemical changes that result in the conversion of muscle to meat (Greaser, 1986; Owens & Meullenet, 2010). One major change in post-mortem muscle is the shift from aerobic to anaerobic metabolism which results in the breakdown of muscle glycogen stores (Owens & Meullenet, 2010). The degradation of muscle glycogen leads to lactate accumulation and a consequent drop in muscle pH (Bendall, 1973; Berri *et al*., 2007; Wang *et al*., 2013). Glycogen content at death is therefore an important factor in determining muscle pH decline (Bendall, 1973; Savenije *et al*., 2002). Furthermore, the rate and extent of post-mortem muscle pH decline is an important determinant of meat quality traits such as colour (Ahn & Maurer, 1990) and tenderness (Bendall, 1973; Jeleníková *et al*., 2008)

Within animal production systems, meat quality is affected by ante-mortem conditions such as level of activity (Casterllini *et al*., 2002; Fanatico *et al*., 2007), physiological stress (Zhang *et al*., 2009) and the genetic makeup of an animal (Berri *et al*., 2005). For example, increased physical activity can lead to decreases in muscle fat content and protein content (Castellini *et al*., 2002; Fanatico *et al*., 2007) while also altering muscle metabolism to become increasingly oxidative (Castellini *et al*., 2002). Physiological stress results in increased ante-mortem glycogen breakdown which decreases glycogen availability at death affecting pH decline and consequent meat quality (Owens & Sams, 2000; Hambrecht *et al*., 2004; Zhang *et al*., 2009). Additionally, meat quality is affected by an animal's genotype due to differences in stress response between genetic strains (Albentosa *et al*., 2003; Fraisse & Cockrem, 2006).

To examine the combined effects of activity level, physiological stress and an animal's genotype on meat quality, laying hen housing systems provide a novel, readily-available model. The predominant laying hen housing system, conventional battery cages (CC), is behaviorally and physically restrictive leading to hen stress (Appleby *et al*., 2002). As a result of these animal welfare concerns, the CC housing system is progressively being banned throughout the world (CEC, 1999; SFS, 1998; SAWO, 1981). Furnished cages (FC) are an alternative housing system to CC that offer the benefits of increased space and amenities to allow for increased natural behavioural expression and physical activity (Appleby, 1998). Hens housed in FC have therefore been shown to exhibit improved bone strength (Jendral *et al*., 2008; Jendral, 2012) and decreased aggression (Jendral, 2012) compared to conventionally-caged hens. In Chapter 2 of this thesis, *Pectoralis major* muscle pH, colour and tenderness of three strains of laying hens housed in either CC or FC systems are investigated in order to determine the effects of housing environment and genetic strain on meat quality.

Although post-mortem muscle pH decline and consequent meat quality is often attributed to glycogenolysis-induced lactate accumulation, there are actually many complex physicochemical factors contributing to the change in muscle pH (Stewart, 1981). In biological systems, $[H^+]$ is dependent on the physicochemical relationships of three systems-independent variables that act within the laws of electrical neutrality, conservation of mass, and mass action equilibrium constants (Stewart, 1981; Stewart, 1983). The three systems-independent variables encompassed within the Stewart model of acid-base regulation include 1) strong ion difference (SID), which is the sum of strong base cations minus the sum of strong acid anions, 2) the total concentration of weak partially-dissociated ions (A_{tot}) and 3) the partial pressure of CO_2 (PCO_2). The Stewart method has been previously validated as an accurate method for determining $[H^+]$ in

various species (Putman *et al.*, 2003; Stämpfli *et al.*, 2006). In Chapter 3 of this thesis, we investigate the three systems-independent variables of the *Pectoralis major* muscles examined in Chapter 2 to understand why differences in muscle pH may or may not be observed under different housing conditions or between different genetic strains. Moreover, we evaluate the accuarcy of the Stewart model for application in meat quality research by comparing measured values of muscle pH to muscle pH calculated using the Stewart model (Stewart, 1981; Stewart, 1983).

1.1 Skeletal Muscle Structure and Function

Skeletal muscle is a highly organized and adaptable organ that represents over 40% of total body mass, and functions in body movement, oxygen consumption and energy metabolism (Zurlo *et al*., 1990; Valberg, 2008). Skeletal muscle consists of multinucleated muscle cells that are often referred to as muscle fibres (Huxley, 1961). Muscle fibre content is enclosed by a plasma membrane, known as the sarcolemma. Many protein channels and pumps that are required for both passive and active movement of ions into and out of the muscle cells are located in the sarcolemma (Fig 1-1) (McComas, 1996; Schiaffino & Reggiani, 2011). This ion transport is essential to overall muscle function. For example, as a nerve impulse reaches a muscle fibre, Na^+ rushes into the cell while K^+ moves out of the cell (Hodgkin & Huxley, 1952). This depolarization causes Ca^{2+} release from an internal membrane system, the sarcoplasmic reticulum, into the sarcoplasm of the cell (Berchtold *et al*., 2000). Calcium then binds to troponin-C which allows the sliding of actin and myosin muscle fibre proteins past one another resulting in a muscle contraction (Fig 1-2) (Berchtold *et al*., 2000).

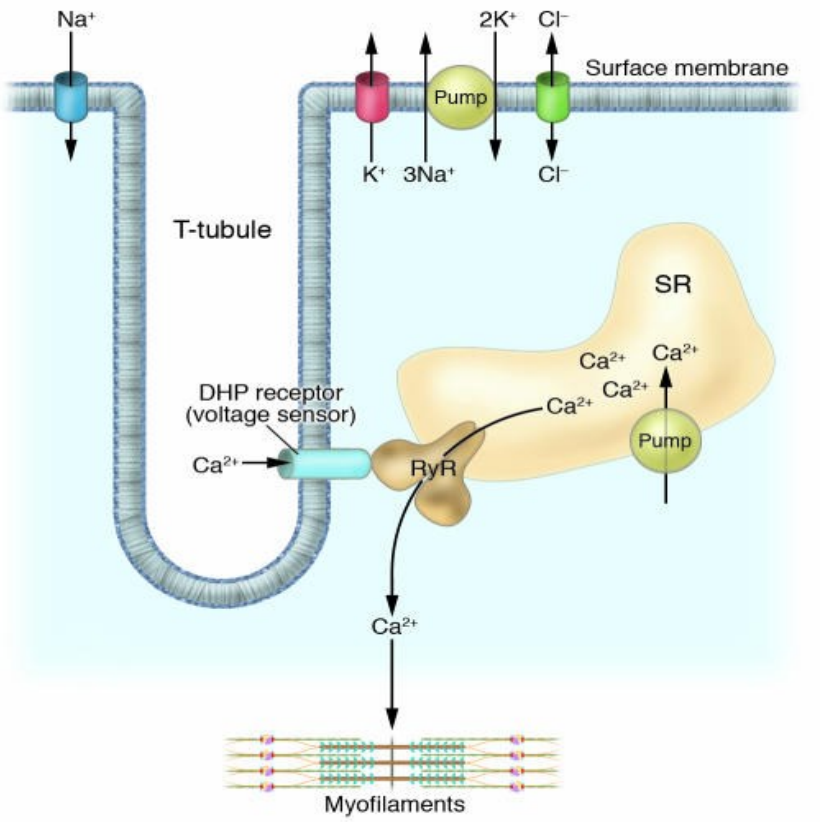

Figure 1-1. Diagram of skeletal muscle ion channels and pumps. *With permission Jurkat-Rott & Lehmann-Horn, 2005.*

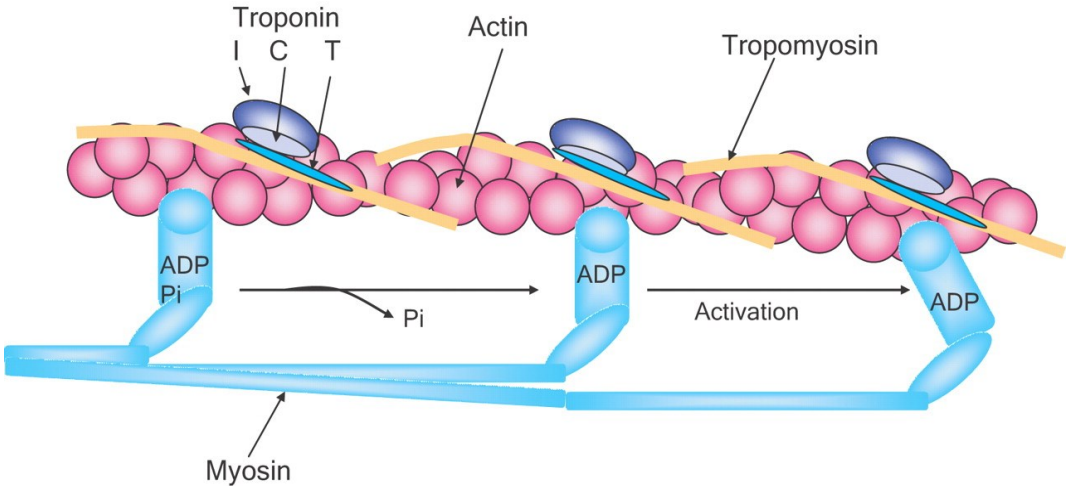

Figure 1-2. Diagram of actin and myosin with cross bridge cycling. *With permission from Hopkins, 2006.*

1.2 Skeletal muscle metabolism

Muscle fibres are classified according to muscle cell contraction speed (fast or slow) and by the type of energy metabolism used by the muscle cells (oxidative or glycolytic) (Brooke & Kaiser, 1970; Pette, 2002). All muscle cell processes require adenosine triphosphate (ATP) as a source of energy (McComas, 1996) and the most immediate source of ATP comes from the degradation of phosphocreatine (PCr) (Westerblad *et al*., 2010). However, skeletal muscle only contains a small reservoir of PCr which is depleted quickly during an increase in energy demand. Therefore, to meet the energy requirements of the muscle, ATP must be generated through alternative methods including oxidative metabolism of fatty acids and carbohydrates, and glycolytic metabolism of muscle glycogen (Westerblad *et al*., 2010).

Anaerobic glycolytic metabolism involves generating ATP through the breakdown of muscle glycogen to pyruvate which is then further broken down to lactate and NAD^+ in order to maintain glycolysis (Westerblad *et al*., 2010). Aerobic metabolism occurs during longer periods of physical activity and involves the production of acetyl-CoA from either oxidation of pyruvate or through β-oxidation of fatty acids (Schiaffino & Reggiani, 2011). Acetyl-CoA enters the Kreb's cycle within the cell mitochondria and results in the production of carbon dioxide, water and a substantial amount of ATP (Schiaffino & Reggiani, 2011).

1.3 Conversion of muscle to meat

In animal production, when an animal is slaughtered, muscle goes through many complex metabolic, physical and biochemical changes resulting in the conversion of muscle to meat (Greaser, 1986). Although blood flow is arrested after death, which prevents oxygen supply, muscle still attempts to maintain ATP levels by following anaerobic metabolism (Owens & Meullenet, 2010). Initially, muscle PCr reserves are depleted to replenish ATP. Once PCr stores

have been exhausted, the main source of ATP is derived from the degradation of glycogen stores (Greaser, 1986). Additionally, during the conversion of muscle to meat, Ca^{2+} is released in the muscle cells and as ATP becomes limited, actin and myosin become permanently attached in a contractile state (Greaser, 1986). This leads to a stiffened state of the muscle referred to as rigor mortis (Greaser, 1986). Rigor is completed when muscle becomes flexible again due to the activity of proteolytic enzymes such as calpains and cathepsins which break down essential muscle proteins (Greaser, 1986). Once the last energy reserves have been depleted and rigor mortis has occurred, the conversion of muscle to meat is complete (Greaser, 1986). For the purposes of this thesis, post-rigor tissue will be referred to as both muscle and meat.

1.3.1 Post-mortem pH Decline

Since blood flow to and from skeletal muscle is arrested after death, metabolic waste cannot be eliminated from the cells (Greaser, 1986). As a result, post-mortem muscle glycogen breakdown leads to lactic acid accumulation (Bendall, 1973; Berri *et al*., 2007; Wang *et al*., 2013). An increase in lactic acid, which dissociates into lactate and H^+, then causes a decline in muscle pH (Bendall, 1973; Berri *et al*., 2007; Wang *et al*., 2013). The amount of muscle glycogen present in the muscle at death is therefore a predictor of the extent to which post-mortem muscle pH will decline (Bendall, 1973; Savenije *et al*., 2002). The rate of pH decline and the ultimate pH (pH_u) reached post-rigor are important factors in determining meat quality (Owens & Meullenet, 2010).

1.4 Meat Quality Characteristics

1.4.1 Meat Colour

Meat colour is the most important factor consumers consider when choosing a meat product as it indicates product freshness and quality (Glitsch, 2000). The colour of meat is determined by a number of factors including concentration of myoglobin, the chemical state of pigments, light reflectance off the meat, and post-mortem muscle pH (Faustman & Cassens, 1990; Mancini & Hunt, 2005). The soluble protein myoglobin is an especially important determinant of meat colour (Mancini & Hunt, 2005). There are various chemical forms of myoglobin that are important in determining muscle colour and each myoglobin form contains an oxygen-carrying heme prosthetic group with a centrally located iron atom (Fe) (Mancini & Hunt, 2005). Myoglobin forms include deoxymyoglobin (Fe^{2+}) which causes a purple-red colour, and oxymyoglobin which causes a bright red colour in fresh meat (Faustman & Cassens, 1990; Mancini & Hunt, 2005). When these two forms of myoglobin oxidize into metmyoglobin (Fe^{3+}), meat appears undesirably brownish-red (Faustman & Cassens, 1990). Muscle pH affects meat color by altering these heme-complex forming reactions of myoglobin (Ahn & Maurer, 1990). A high muscle pH (> 6.2) has been shown to favor heme-complex-forming reactions of myoglobin and hemoglobin, thereby increasing meat redness (a*) (Ahn & Maurer, 1990). In contrast, a lower pH (< 6.0) favors protein denaturation which enhances light scattering and leads to increased meat lightness (L*) (Swatland, 2008).

1.4.2 Sensory Characteristics

1.4.2.1 *Water Holding Capactiy (WHC)*

Water holding capacity (WHC) is another important meat quality characteristic as meat tenderness, juiciness and appearance are all affected by the ability of muscle to retain water (Owens & Meullenet, 2010). Most water inside muscle tissue is located within the intracellular spaces between actin and myosin filaments (Offer & Knight, 1988). The ability for meat to retain water within these intracellular spaces is influenced by many factors including pH, ionic strength, and development of rigor mortis (Offer & Knight, 1988). Post-mortem muscle pH decline causes protein denaturation and loss of protein solubility therefore reducing WHC (Offer & Knight, 1988) and past studies have shown a positive correlation between muscle ultimate pH and meat WHC (Barbut, 1993; Qiao *et al*., 2001). Muscle WHC is commonly measured though drip loss, defined as the amount of water lost from the post-mortem muscle and through cook loss, the amount of water lost in the meat throughout cooking (Huff-Lonergan & Lonergan, 2005).

1.4.2.2 *Meat Tenderness*

Tenderness is also an important meat quality characteristic for consumer acceptance (Owens & Meullenet, 2010). Tenderness or toughness of meat is the result of post-mortem structural changes in the muscle (Owens & Meullenet, 2010). Post-mortem muscle changes such as rigor development, pH decline and structural protein alteration all have significant effects on meat tenderness and consequent meat quality (Owens & Meullenet, 2010). M-calpain, a proteolytic enzyme, is activated in the muscle post-mortem leading to muscle protein breakdown which ultimately affects meat tenderness (Huff-Lonergan *et al*., 2010). Additionally, muscle proteins such as actin and myosin become oxidized in the initial stages of post-mortem when

reactive oxygen species are still present in the cell, decreasing meat tenderness (Huff-Lonergan *et al*., 2010). Since pH affects the enzymatic activity in muscle protein breakdown, there is also a relationship between muscle pH and tenderness (Bendall, 1973; Jeleníková *et al*., 2008). For example, meat pH_u values that fall within 5.9 - 6.1 typically yields optimal meat tenderness (Van Laack *et al*., 2000; Barbut *et al*., 2005), while pH values below 5.9 typically yield increasingly tender meat and pH values above 6.1 yield tougher meat (Barbut *et al*., 2005; Qiao *et al*., 2001).

1.5 Ante-mortem Factors Affecting Meat Quality

In animal production, meat quality traits can be affected by a number of ante-mortem factors including physiological stress (Owens & Sams, 2000; Zhang *et al*., 2009) and the level of activity throughout an animal's life (Castellini *et al*., 2002; Fanatico *et al*., 2007; Branciari *et al*., 2008). Moreover, meat quality can be affected by biological factors such as the genetic makeup of an animal (Fanatico *et al*., 2007; Berri *et al*., 2005), an animal's gender (Bianchi *et al*., 2007), and the age of an animal at slaughter (Bianchi *et al*., 2007). In this thesis, the effects of housing activity level, ante-mortem stress and the genetic makeup of an animal on meat quality are discussed.

1.5.1 Effects of Physical Activity on Meat Quality

Level of activity has been shown to affect various meat quality traits (Castellini *et al*., 2002; Fanatico *et al*., 2007). Fanatico *et al*. (2007) found that chickens raised with outdoor access had lower muscle pH_u, increased water loss through cooking and increased protein percentage as compared to conventionally-raised birds. Similarly, Castellini *et al*. (2002), compared meat quality of conventionally-housed chickens and chickens raised organically with access to a grass paddock. The increased activity of organically raised birds led to reduced abdominal fat, increased muscle development, decreased muscle pH_u and increased muscle

WHC, decreased lipid percentage and increased total iron and heme-iron (Castellini *et al*., 2002). Increases in activity can induce a muscle fibre type transition from glycolytic fibre types toward increasingly oxidative muscle fibres (Branciari *et al*., 2008). Therefore, the increase in muscle heme-iron demonstrated by Castellini *et al*., (2002) may have resulted from muscle metabolism that was altered toward oxidative metabolism due to the elevated energy demand in the organic housing system.

1.5.2 Effects of Physiological Stress on Meat Quality

Under conditions of physiological stress, two main physiological responses occur. The "fight or flight" response activates the sympathetic adrenal medulla axis (SAM) which ultimately leads to the secretion of catecholamine hormones from the adrenal gland medulla (Cannon, 1929). The second response involves activation of the hypothalamic-pituitary-adrenal (HPA) axis which ultimately leads to the release of glucocorticoid hormones from the adrenal cortex (Selye, 1950). These hormones are considered metabolic as they increase energy demand, causing increases in whole body metabolism and blood glucose levels (Moberg, 2000).

Muscle metabolism is increased during stress due to the increased energy demand. To mobilize energy during stress, glucocorticoids increase the activity of the enzymes involved in muscle metabolism (Bodine & Furlow, 2015). As a result, muscle glycogen breakdown occurs (Hambrecht *et al*., 2004; Zhang *et al*. 2009). Ante-mortem stress therefore decreases muscle glycogen availability at death (Zhang *et al*., 2009) which affects the extent of post-mortem muscle pH decline and consequent meat quality (Owens & Sams, 2000; Hambrecht *et al*., 2004; Zhang *et al*., 2009). Past studies have shown that ante-mortem stress results in a high meat pH_u which affects meat colour, WHC and tenderness (Owens & Sams, 2000; Hambrecht *et al*., 2004). Owens and Sams (2000) found that long-term transport stress for broiler chickens resulted in

higher muscle pH_u and darker meat than a non-transported group. Similarly, Hambrecht *at al*., (2004) found that subjecting pigs to pre-slaughter transport stress resulted in higher muscle pH_u, and darker meat compared to an unstressed control group.

1.5.3 Meat Quality and the Genetic Makeup of an Animal

Meat quality can be affected by the genetic makeup of an animal. Animals of different genetic strains are known to have varying physiological and behavioural responses to stress (Zavy *et al*., 1992; Albentosa *et al*., 2003; Fraisse & Cockrem 2006). For example, fear response and corticosteroid levels are known to differ across genetic strains in both mammalian and avian species (Zavy *et al*., 1992; Albentosa *et al*., 2003; Fraisse & Cockrem, 2006). Zavy *et al*. (1992) showed higher post-handling cortisol levels for Brahman-Cross cattle as opposed to English crosses. Albentosa *et al*. (2003) showed increased duration of tonic immobility, a common fear response for laying hens, in White Leghorn strains compared to ISA Brown, Columbian Blacktail and Ixworth laying hens (Albentosa *et al*., 2003). Increased corticosterone levels and longer durations of tonic immobility have also been found for White Leghorns compared to Brown Hyline hens after handling (Fraisse & Cockrem, 2006). Since stress has been shown to affect meat quality and there are genetic differences in the ability to cope with stress, it can be expected that meat quality will be affected by the genetic makeup of an animal as well.

1.6 Meat Quality Misconceptions

Although the post-mortem muscle pH decline is commonly attributed to glycogenolysis-induced lactate accumulation (Bendall, 1973; Berri *et al*., 2007; Wang *et al*., 2013), previous studies have shown that muscle pH, lactate content and glycogen content are not always correlated (Zhang *et al*., 2007; Matarneh *et al.*, 2015; England *et al.*, 2016). For example, Matarneh *et al*. (2015) showed that although pigs with the AMPK$\gamma 3^{R200Q}$ mutation for increased

muscle glycogen content had lower muscle pH_u than wild-type pigs, both pig strains had similar muscle lactate content. In this study, the greater muscle pH decline for AMPK$\gamma3^{R200Q}$ pigs was caused by a combination of lactate accumulation and lower muscle buffering capactiy (Matarneh *et al*., 2015). Research that fails to show correlations between muscle pH and lactate content suggests that there are factors other than post-mortem muscle glycogenolysis and resultant lactate accumulation that affect muscle pH decline and consequent meat quality (Monin & Sellier, 1985; Zhang *et al*., 2009, England *et al*., 2016; Matarneh *et al*., 2015).

1.7 The Stewart Model of Acid-Base Regulation

Stewart (1981; 1983) proposed a comprehensive, quantitative approach to determine the complex physical chemical (physicochemical) relationships that control pH within biological systems. The Stewart Model of acid-base regulation provides an accurate account of the factors affecting muscle $[H^+]$ and has been validated across various mammalian and avian species (Putman *et al*., 2003; Lindinger *et al*., 2005; Stämpfli *et al*., 2006). Following the physicochemical laws of electrical neutrality, conservation of mass, and mass action equilibrium constants, the Stewart method specifies that a change in the dependent variable, $[H^+]$, is determined by the relationships of three systems-independent variables (Stewart, 1981; Stewart, 1983). The three systems-independent variables that determine muscle $[H^+]$ are the strong ion difference (SID), the total concentration of partially dissociated nonvolatile acids (A_{tot}), and the partial pressure of carbon dioxide (PCO_2) (Stewart, 1981; Stewart, 1983).

1.7.1 SID

The strong ion difference ([SID]) is the sum of strong base cation concentrations minus the sum of strong acid anion concentrations ([SID] = Σ[strong cations] – Σ[strong anions]) (Stewart, 1981; Stewart, 1983). Strong base cations and strong acid anions refer to ions that are

fully dissociated in physiological solutions (Stewart, 1983; Jones, 1990; Lindinger, 1995). The dissociation constants (K_A) for strong acids are greater than 10^{-4} and for strong bases are lower than 10^{-12} (Jones, 1990). Therefore, the most common strong base cations in biological systems are K^+, Na^+, Ca^{2+}, and Mg^{2+} and the most common strong acid anions are Cl^- and lactate (Lac^-) (Stewart, 1983). Electrical neutrality requires that a solution of strong ions follows this equation:

$$[SID] + [H^+] - [OH^-] = 0 \quad (1)$$

An inverse relationship exists between $[H^+]$ and [SID] (Stewart, 1983). This means that when strong anion concentrations are high, [SID] is decreased, resulting in increased $[H^+]$. In contrast, as strong cation concentration increases, [SID] increases, leading to decreases in $[H^+]$ (Stewart, 1983).

1.7.2 A_{tot}

The total concentration of partially dissociated nonvolatile weak acids that act as weak acid buffers is termed $[A_{tot}]$ (Stewart, 1981; Stewart, 1983). The K_A for these compounds falls between 10^{-4} and 10^{-12} and weak acids with a K_A around neutral 10^{-7} typically act as buffers (Jones, 1990). These nonvolatile weak acids (HA) include phosphorylated metabolites, phosphates and soluble proteins (Lindinger, 1995) and partially dissociate into H^+ and A^- ions (Stewart, 1983). Therefore, increases in $[A_{tot}]$ lead to increases in $[H^+]$. The following equation for the dissociation equilibrium of $[A_{tot}]$ must be satisfied:

$$[H^+] \times [A^-] = K_A \times [HA] \quad (2)$$

Therefore, $[A_{tot}]$ must include the undissociated weak acids and dissociated anions, $[A^-]$ such that:

$$[A^-] + [HA] = [A_{tot}] \quad (3)$$

Following electrical neutrality, in a biological system that includes both strong and weak acids, the following is required:

$$[SID] + [H^+] - [A^-] - [OH^-] = 0 \qquad (4)$$

1.7.3 P_{CO_2}

The presence of CO_2 in a biological system is identified as the partial pressure of CO_2 (P_{CO_2}) in the Stewart model (Stewart, 1983). Due to the following reversible reaction (Jones, 1987), increases in P_{CO_2} leads to increases in $[H^+]$:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \qquad (5)$$

Following electrical neutrality, biological systems that include strong and weak ions and CO_2 satisfy the following equation:

$$[SID] + [H^+] - [HCO_3^-] - [A^-] - [CO_3^{2-}] - [OH^-] = 0 \qquad (6)$$

1.7.4 The Stewart Equation

Taken together, the above equations for each of the three systems-independent variables (Eq. 1-6) and the specific parameters for dissociation of weak acids (K_A), water dissociation equilibrium (K'_W), formation of bicarbonate ion (K_C) and formation of carbonate ion (K_3) can be applied to solve for $[H^+]$ simultaneously in one equation:

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_{tot}]) - (K_C P_{CO_2} + K'_W)\} \qquad (7)$$
$$\times [H^+]^2 - \{K_A(K_C P_{CO_2} + K'_W) + (K_3 K_C P_{CO_2})\}[H^+] - (K_A K_3 K_C P_{CO_2}) = 0$$

The mass action equilibrium constants are as follows: (i) $K_A = 3.0 \times 10^{-7}$ Eq/L; (ii) $K'_W = 4.4 \times 10^{-14}$ (Eq/L)^2; (iii) $K_C = 2.58 \times 10^{-11}$ (Eq/L)^2; (iv) $K_3 = 6.0 \times 10^{-11}$ Eq/L.

Solving for this equation is not feasible by hand. Therefore, computer programs have been created in order to calculate [H^+] using the Stewart method (Stewart, 1983; Jones, 1990). These computer programs calculate [H^+] from the three systems-independent variables and the parameters specific to what is being measured. It is also possible to calculate the contributions of each of the systems-independent variables to [H^+] using this computer program.

1.8 Using Laying Hen Housing to Assess the Effects of Physical Activity, Physiological Stress and an Animal's Genotype on Meat Quality

Laying hen housing provides a unique, valuable and readily-available model to assess the effects of activity level, physiological stress and the genetic makeup of an animal on meat quality in animal production. Laying hens are commonly housed in conventional battery cages that restrict the expression of natural behavioural and physical movement (Appleby *et al*., 2002). Such restrictions lead to a lifetime of chronic stress and inactivity (Yue & Duncan, 2003). By comparison, furnished cages afford hens increased space and amenities that allow for natural behavioural expression and load-bearing activity (Appleby, 1998). This research therefore uses a unique model comparing laying hen housing systems to provide novel information regarding the combined influences of physical activity, physiological stress and an animal's genotype on laying hen *Pectoralis major* meat quality.

1.8.1 Cage Housing for Laying Hens

1.8.1.1 *Conventional Battery Cages*

Worldwide, conventional battery cages (CC) are the predominant laying hen housing system. Conventional cage systems facilitate animal management due to the automation of feeding, watering, egg collection and manure removal (Appleby, 2003). However, CC are

severely restrictive in behavioural expression and physical movement (Appleby *et al*., 2002) which affects hen overall health and well-being (Yue & Duncan, 2003; Jendral *et al*., 2008). As a result, many countries have banned or have begun to phase out CC laying hen housing (CEC, 1999; SFS, 1998; SAWO, 1981).

Laying hens housed in CC experience stress due to the behavioral and physical restriction (Appleby *et al*., 2002). The inability to perform highly motivated behaviours such as nesting, dust bathing and perching leads to frustration and distress (Dawkins, 1990). Due to behavioural restriction, increased expression of stereotypical behaviour also occurs in CC, further indicating stress (Yue & Duncan, 2003). Physical restriction in both cage height and cage overall area also prevents activity in CC (Nicol, 1987; Appleby *et al*., 2002). Space restriction and the inability to express load-bearing activity in CC can lead to metabolic disorders such as osteoporosis (Wilson *et al*., 1992; Jendral *et al*., 2008) and high instances of bone fractures (Gregory & Wilkins, 1989). Weak bones often splinter during processing, affecting laying hen meat quality (Gregory & Wilkins, 1989). Laying hen meat is typically not processed for human consumption for this reason and because meat yield (Damme & Ristic, 2003) and meat tenderness (Rizzi *et al*. 2007) are lower than broiler chickens.

1.8.1.2 *Furnished Cages*

Furnished cages (FC) offer an alternative to CC as they maintain the production benefits of CC, but also include amenities which allow natural nesting, perching, foraging and dust bathing behaviours (Appleby, 1998) and provide increased cage height which encourages load-bearing activity (Nicol, 1987). The ability to perform load-bearing activity results in improved bone health for hens housed in FC compared to conventionally-caged hens (Jendral *et al*., 2008, Jendral, 2012) and may also reduce the onset of osteoporosis (Jendral, 2012). Additionally, due

to the increased expression of natural behaviours, hens housed in FC may experience reduced stress compared to hens housed in CC (Appleby *et al*., 2002). Past research has shown that hens housed in FC exhibit reduced aggressive pecking behaviours (Jendral, 2012) indicating that these hens are less frustrated than hens housed in CC. Increased bone health and overall reduced stress could benefit laying hen meat quality by reducing bone splintering and improving overall muscle health.

Although FC provide increased physical space and allow for the expression of highly motivated behaviours, they also house larger group sizes than CC. Laying hens naturally form a complex, hierarchal social system (Estevez *et al*., 1997). In confined cage systems, laying hen social structure is best maintained in smaller groups of up to 20 hens (Keeling *et al*., 2003). Increasing group size within cage systems may prevent hens from establishing and maintaining a social hierarchy, which results in aggression and resultant social stress (Keeling *et al*., 2003). However, when group size is too large for individual recognition, hens may no longer attempt to establish dominance through increased aggression (Estevez *et al*., 1997; Keeling *et al*., 2003). Under these conditions, social tolerance may occur and aggression and social stress are reduced (Estevez *et al*., 1997).

1.8.2 Laying Hen Genetic Strains

The laying hen strains examined in this thesis originate from commercial laying hen stocks that were selected for egg laying efficiency and quality. These strains are common among laying hen producers and include Shaver White (SH), Lohmann Lite (LL) and Lohman Brown (LB). The SH strain (ISA, Hendrix Genetics Company) is a white hen and is considered a light hybrid, weighing 1.65 kg on average (Shaver White, ISA Management Guide). The LL strain (Lohmann LSL-Lite, Lohmann Tierzucht) is also a white hen and considered a light hybrid,

weighing 1.75 kg on average. The LB strain (Lohmann Brown Classic, Lohmann Tierzucht) is a brown hen and is considered a medium hybrid, weighing 2.0 kg on average (Lohmann LSL-Lite, and Lohmann Brown Classic Management Guides).

1.9 Thesis Objectives

The goal of the research presented in Chapter 2 was to evaluate the effects of activity level, physiological stress and genetic strain on meat quality using traditional production measures. Therefore, muscle pH, colour and tenderness were assessed for three strains of laying hens (Shaver White, Lohmann Lite, Lohmann Brown) housed in conventional (CC) and furnished cage (FC) systems. It was hypothesized that hens housed in FC who have greater opportunity for movement and the ability to express highly motivated behaviour would exhibit improved meat quality compared to conventionally-caged hens, and that Lohmann Brown hens may experience a lower stress response than Shaver White and Lohmann Lite hens improving overall meat quality for this strain.

Due to common misconceptions surrounding the factors contributing to post-mortem muscle pH decline and consequent meat quality, a second study was undertaken (Chapter 3). The purpose of this study was to determine muscle glycogen content and the three systems-independent variables that affect muscle $[H^+]$ according to the Stewart model (Stewart, 1981; Stewart, 1983). Additionally, the accuracy of the Stewart model in meat quality research was determined through a comparison of measured muscle $[H^+]$ values to values of muscle $[H^+]$ as calculated using the Stewart equation (eq. 7). It was hypothesized that the three systems-independent variables would differ between genetic strains and between housing environments. Furthermore, it was hypothesized that differences in the three systems-independent variables would account for variations in measured muscle $[H^+]$.

CHAPTER 2: Effect of housing environment on laying hen meat quality: Assessing *Pectoralis major* pH, colour and tenderness in three strains of 80 to 81 week old laying hens housed in conventional and furnished cages

2.1 Introduction

This research was conducted as part of a larger, longitudinal study examining the physiological and behavioural health, welfare, and productivity of laying hens housed in conventional and furnished cage systems. Indices of physiological stress, behaviour, condition, bone health, egg production and egg quality are examined elsewhere (Jendral, 2012). This chapter focuses on the effects of housing environment on laying hen meat quality.

Biochemical and physical changes that take place during post-mortem muscle metabolism influence meat quality (Bendall, 1973). Post-mortem muscle metabolism follows anaerobic glycolysis and involves the conversion of muscle glycogen to lactate (Bendall, 1973). The resultant breakdown of adenosine triphosphate (ATP) and accumulation of lactate induce a decline in muscle pH (Bendall, 1973; Wang *et al*., 2013). The extent of post-mortem muscle metabolism, and consequent decline in muscle pH, is dependent in part upon muscle glycogen availability at death (Savenije *et al*., 2002), and the resultant ultimate pH determines muscle colour (Ahn & Maurer, 1990) and tenderness (Bendall, 1973; Jeleníková *et al*., 2008). Typically, a muscle pH greater than 6.2 is associated with darker and firmer meat (Ahn & Maurer, 1990; Barbut *et al*., 2005; Jeleníková *et al*., 2008; Quio *et al*., 2011). In contrast, a muscle pH lower than 5.8 is typically associated with paler and softer meat (Ahn & Maurer, 1990; Barbut *et al*., 2005; Quio *et al*., 2011). Normal muscle colour and tenderness values generally occur when ultimate pH ranges between 5.9 – 6.1 (Van Laack *et al*., 2000; Barbut *et al*., 2005; Quio *et al*., 2011).

Post-mortem muscle metabolism, and consequent meat quality, is affected by pre-slaughter stress. During periods of stress, energy demand is elevated (Selye, 1950), resulting in ante-mortem muscle glycogen breakdown (Moberg, 2000). Ante-mortem stress therefore decreases glycogen availability at death (Zhang *et al*., 2009), affecting the extent of muscle pH decline and consequent meat quality. Owens and Sams (2000) found that birds who were exposed to long-term transport stress had darker meat and higher muscle ultimate pH than non-transported birds. The researchers attributed reduced meat quality of transported birds to stress-induced ante-mortem decreases in muscle glycogen content (Owens & Sams, 2000). Zhang *et al*. (2009) subsequently provided evidence of decreased muscle glycogen content in broiler chickens subjected to long-term transport stress compared to an unstressed control group.

The ability to cope with stress appears to be determined, in part, by physiological and behavioural traits (Buitenhuis *et al*., 2003). Albentosa *et al*. (2003) studied the fear response of White Leghorn, ISA Brown, Columbian Blacktail and Ixworth laying hens by measuring tonic immobility. The authors found that White Leghorns had longer durations of tonic immobility, providing evidence that these birds were more fearful than the other strains (Albentosa *et al*., 2003). Additionally, Fraisse and Cockrem (2006) discovered higher corticosterone levels and longer durations of tonic immobility in White Leghorn hens compared to Brown Hyline hens after handling. These studies provide evidence of differences in stress response among genetic strains of laying hens.

Worldwide, laying hens are predominantly housed in conventional cages (CC). Laying hens housed in CC experience stress due to inability to express highly motivated behaviours (Appleby *et al*., 2002). This behavioural limitation leads to frustration which has been shown

through increased expression of stereotypical behaviours (Yue & Duncan, 2003). Limited physical space, both in cage depth and cage height also prevents activity in CC (Appleby *et al*., 2002; Nicol, 1987). This overall space restriction in CC leads to metabolic disorders such as osteoporosis (Jendral *et al*., 2008; Wilson *et al*., 1992) and other systemic conditions that might also affect meat quality.

Due to the welfare concerns of conventional layer housing, various countries have banned or have begun to phase out the use of conventional cages (CEC, 1999; SFS, 1998; SAWO, 1981). Furnished cages (FC) offer an alternative to CC as they contain amenities that facilitate the expression of highly motivated behaviours, such as nesting, perching, dust bathing and foraging (Appleby, 1998). In addition, furnished cages provide increased cage height which allows for more bone loading activities such as jumping, wing flapping and wing-leg stretching (Nicol, 1987). Therefore, hens housed in furnished cages experience improved bone health (Jendral *et al*., 2008) and may experience reduced stress compared to conventionally-caged hens.

Despite the behavioural and physiological benefits conferred by the increased space availability and access to cage amenities, FC typically house larger group sizes than CC. This may contribute to hen stress (Keeling *et al*., 2003; Bilcik & Keeling, 1999) since chickens naturally form social hierarchies that are best established and maintained in smaller groups (Estevez *et al*., 1997). Increasing group size may prevent hens from establishing and maintaining a social hierarchy resulting in increased aggression and consequent social stress (Keeling *et al*., 2003). It is possible however, that laying hens in very large groups may attempt to avoid repeated aggressive encounters by engaging in social tolerance (Estevez *et al*., 1997). Under such circumstances, increasing group size might not increase social stress (Estevez *et al*., 1997).

Laying hen meat is typically not processed for human consumption for a number of reasons. Laying hens have lower meat yield (Damme & Ristic, 2003) and less tender meat than broiler chickens (Rizzi *et al*., 2007). Additionally, fragile and fractured bones often splinter throughout muscle tissue during processing, further affecting laying hen meat quality (Gregory & Wilkins, 1989). For hens housed in FC, increased locomotory activity and improved bone health may benefit muscle integrity and decrease bone fracture occurrences thereby improving meat quality.

The objective of this study was to evaluate the effects of laying hen housing environment and strain differences on meat quality. We assessed muscle pH, colour, and shear force of *Pectoralis major* muscles obtained from 80 to 81 week old Shaver White, Lohmann Lite, and Lohmann Brown laying hens housed in conventional and furnished cage systems. We hypothesized that hens in FC, who had greater opportunity to express highly motivated and locomotory behaviour, would exhibit improved meat quality over conventionally-caged hens despite the social stress resulting from group size in FC. We further hypothesized that meat quality would differ among laying hen strains.

2.2 Materials and Methods

2.2.1 Animals and Experimental Design

All research was carried out in accordance with the guidelines of the Canadian Council for Animal Care. Research was authorized by the Animal Care and Use Committee at the Nova Scotia Agricultural College (NSAC), where the animals were housed, and by the Animal Care and Use Committee for Livestock at the University of Alberta, where flock one laboratory analyses were conducted.

Two consecutive laying hen flocks consisting of 280 Shaver White (SH), 280 Lohmann Lite (LL), and 280 Lohmann Brown (LB) hens were housed at the Atlantic Poultry Research Institute (APRI) at the NSAC. The SH hens were a light, white, ISA hybrid, while LL hens were a light, white, Leghorn hybrid and LB hens were a medium, brown Leghorn hybrid (Shaver White, Lohmann LSL-Lite, and Lohmann Brown Classic Management Guides). Each hen was randomly assigned to either a conventional cage (CC) or a furnished cage (FC) upon arrival at the facility at either 18 weeks (flock 2) or 19 weeks (flock 1). Birds were housed at 23^{o}C in both systems and length of daylight was gradually increased from 11.5 to 15 hours per day by 25 weeks of age.

The conventional cage (CC) system consisted of nine mobile batteries. Each battery contained two tiers of four cages totaling 72 conventional cages, or 24 cages per strain. Each CC measured 60 cm wide by 55 cm deep and had a height of 45 cm. Five hens were housed in each CC, allowing 660 cm^2 of floor space per hen.

The furnished cage (FC) system consisted of 12 furnished battery cages, providing four cages per strain. Each FC measured 240 cm wide by 110 cm deep and had a height of 50 cm. Forty hens were housed in each FC, allowing 660 cm^2 of usable floor space per hen. Additionally, a nest box, perches, and a dust bath were present in each FC. The nest box was lined with artificial turf and was 60 cm wide by 55 cm deep, providing each hen with an additional 92 cm^2 of space. The raised, metal dust bath which measured 60 cm wide and 20 cm deep, was opened daily 8 hours after lights on and filled with a substrate consisting of a mixture of wood shavings and feed. Dust baths were closed nightly 45 minutes prior to lights out to prevent dust bath egg laying during the dark period. Additionally, each FC contained three

hardwood, semi-circular perches which measured 5 cm wide and 2.5 cm deep and extended the length of the cage.

2.2.2 Processing and Sample Preparation

Between 80 and 81 weeks, 25 randomly selected hens from each FC and all hens from one randomly selected CC per strain, per battery were prepared for processing. Hens were gently removed from their cages, weighed, scored for feather condition, palpated for fractures using a palpation method developed and verified in our laboratory (Jendral, 2012), and carefully transported to the processing facility at the APRC. To minimize acute stress during transportation, the following measures were taken: feed and water were made available up to the time of transport, lights were dimmed during handling, transportation crates were lined with clean, soft bedding to prevent injury, meal worms were scattered on the floor of the crates, and crate stocking density was limited to 5 hens per crate. At processing, hens were gently and individually removed from the transportation crates and carefully positioned upside down in foothold shackles. Only 5 hens were processed at a time to further minimize time between shackling and stunning. Hens were rapidly rendered unconscious via electrical stun and the right jugular artery was immediately severed. Hens were bled out for 90 sec, and were then placed in a scalder at 57°C for two minutes and a feather plucker for 45 sec. Hens were then re-palpated for post-processing bone fractures. Following the method of Jendral *et al*. (2008) humeral bone breaking strength was determined on three randomly selected hens from each CC and FC using a TA.XT Plus Texture Analyzer (Stable Micro Systems, Texture Technologies Corporation, NY, USA). Palpation measures conducted prior to, and following, processing and humeral bone breaking strength (BBS) measures were used to correlate bone fracture data with muscle analyses.

At 17 minutes post-mortem, 2.5 cm cube muscle samples were extracted from the right superior *Pectoralis major* muscle and snap frozen in liquid nitrogen. Carcasses were stored at 4°C for 24 h, after which the entire left *Pectoralis major* was excised and stored at -20°C until further analysis. Samples from flock 1 were subsequently transported on dry ice to the University of Alberta where they were stored at -80°C until further analysis.

2.2.3 pH Measurements

Initial pH was determined on 17 min post-mortem right *Pectoralis major* samples following a modification of the homogenate method of Jeacocke (1977). Enzymatic activity was arrested by homogenizing 0.5 g of muscle tissue in 5 mL of cold 5 mM iodoacetate buffer for 10 sec using a Polytron homogenizer (Brinkman Instruments, Rexdale, ON). Tubes were inverted three times and pH was measured directly on the homogenate (Accumet Basic AB15, Fisher Scientific). Ultimate pH was determined on 2.5 cm cube muscle samples isolated from the superior portion of the 24 h post-mortem left *Pectoralis major* muscle following the identical procedure as initial pH, but using MilliQ water for homogenization in place of the iodoacetate buffer.

2.2.4 Colour Measurements

Muscle colour measures were conducted posterior to the point of cube extraction on the right *Pectoralis major* muscle at 30 min post-mortem. Duplicate colour measures were performed using a MiniScan XE Plus colorimeter (Hunter Associates Laboratory, Reston, VA, USA) and the International Commission of Illumination (CIELAB) system for lightness (L*), redness (a*), and yellowness (b*).

2.2.5 Shear Force Measurements

Left *Pectoralis major* muscle samples were thawed for 24 h at 4°C. Sample bags were suspended in either a water bath (Flock 1; Fisher Scientific Hi-Temp Water Bath model 160, Fisher Scientific, USA) or in a double jacketed kettle (Flock 2; Groen model 55-50, Groen Mfg. Co, Elk Grove Village, Illinois, USA) at 80°C for 20 min. All samples were then cooled to room temperature and stored at 4°C for 24 h. Shear force was determined following the razor blade shear method of Cavitt *et al*. (2004) using an Instron 4443 Universal Testing System with Merlin Series IX Software with a 1 kN load cell for flock 1 samples and a TA.XT Plus Texture Analyzer (Stable Micro Systems, Texture Technologies Corporation, NY, USA) with Version 6.0.2.0 integrated software and a 10 kg load cell for flock 2 samples. Texture analyzers for both flocks were adapted using chisel edge blades (#17, 9.5 mm, Excel Hobby Blades Corp., NJ, USA) with a crosshead speed set at 5 cm/min. To accommodate for variation in breast size, the depth of each muscle sample was measured prior to shear force analysis, for each cut in flock 1 and for the first cut in flock 2. Blade penetration depth was set at 90% of the sample breast depth. Cuts were made in triplicate, 1 cm apart, and blades were replaced every 100 cuts.

2.2.6 Statistical Analysis

Response variables were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, 2013) to assess treatment, strain, and treatment by strain interaction differences. *Pectoralis major* muscle depth was used as a covariate for the muscle pH, colour, and shear force analyses. Data are reported as least square means and are compared using the least significant differences test for planned comparisons. The level of significance for all analyses was assessed at $P < 0.05$.

Coefficients (r) for correlating pH (initial pH, ultimate pH and pH decline) with colour (L*, a* and b*) and shear force, and shear force with humeral bone breaking strength, pre-slaughter proportions of broken bones and post-slaughter proportion of broken bones, were calculated using Pearson correlations (SAS Institute, 2013). Correlation calculations were conducted with all treatment groups pooled, to determine overall relationships, as well as for individual housing treatments, to examine relationships within each treatment group, and for individual strains within a treatment.

2.3 Results

2.3.1 Body Weight (BW)

For both flocks, when strains were combined, BW between 80 and 81 weeks was higher in CC than in FC ($P < 0.05$) (Table 2-1). When treatments were combined, BW was higher for LB than both LL ($P < 0.0001$) and SH ($P < 0.0001$), and was higher for LL than SH ($P < 0.05$) (Table 2-1).

Housing treatment differences were observed for individual strains. Flock 1 SH BW was higher in CC than in FC ($P < 0.05$) (Table 2-1), and in flock 2, LB BW was higher in CC than in FC ($P < 0.0001$) (Table 2-1). Within a housing treatment, strain differences were observed. Within CC, BW was higher for LB than both LL ($P < 0.0001$) and SH ($P < 0.0001$) (Table 2-1) for both flocks, and in flock 2, BW was higher for LL than SH ($P < 0.05$) (Table 2-1). Additionally, in both flocks, FC BW was higher for LB than both LL ($P < 0.0001$) and SH ($P < 0.0001$) and in flock 1, FC BW was higher for LL than SH ($P < 0.001$) (Table 2-1).

2.3.2 Breast Muscle Depth (BD)

When strains were combined, BD did not differ between housing treatments in either flock 1 or flock 2 (Table 2-1). When treatments were combined, BD did not differ between strains in flock 1 (Table 2-1). In flock 2 however, SH had a higher BD than LL ($P < 0.01$) and LB ($P < 0.05$) (Table 2-1). Additionally, within a housing treatment, strain differences were observed in flock 2**.** Within CC, SH had a higher BD than LL ($P < 0.05$) and LB ($P < 0.05$) (Table 2-1) and within FC, SH had a higher BD than LL ($P < 0.05$) (Table 2-1).

2.3.3 Muscle pH

For both flocks, when strains were combined, there were no housing treatment differences in muscle initial pH (pH_{17}), ultimate pH (pH_u) or pH decline (ΔpH) (Tables 2-2 & 2-3). When treatments were combined, flock 1 muscle pH_{17} and pH_u were lower for LB than SH ($P < 0.001$) and LL ($P < 0.001$) (Table 2-2), and ΔpH was lower for SH than LL ($P < 0.05$) and LB ($P < 0.05$) (Table 2-2). When treatments were combined in flock 2, there were no differences in muscle pH_{17} and ΔpH between strains, however muscle pH_u was lower for LB than SH ($P < 0.05$) and LL ($P < 0.05$) (Table 2-3).

Housing treatment differences were not observed for individual strains for pH_{17}, pH_u or ΔpH in flock 1 (Table 2-2), or for pH_{17} or ΔpH in flock 2 (Table 2-3). However, Flock 2 SH muscle pH_u was higher in FC than in CC ($P < 0.05$) (Tables 2-2 & 2-3). Strain differences were observed within housing treatments. Within CC, flock 1 muscle pH_{17} and pH_u were lower for LB than SH ($P < 0.05$) and LL ($P < 0.01$) (Table 2-2). Within FC, flock 1 muscle pH_{17} and pH_u were lower for LB than SH ($P < 0.05$) and LL ($P < 0.05$) (Table 2-2). Additionally, flock 1 ΔpH was lower for FC SH than FC LL ($P < 0.05$) (Table 2-2). In flock 2, no strain differences were

observed within a housing treatment for pH_{17} or ΔpH, however muscle pH_u was lower for FC LB than FC SH ($P < 0.05$) (Table 2-3).

2.3.4 Muscle Colour

For both flocks, when strains were combined, muscle L* and b* did not differ between housing systems (Tables 2-4 & 2-5). Muscle a* however, was higher in CC than FC for both flocks ($P = 0.05$) (Tables 2-4 & 2-5). When treatments were combined, no strain differences were observed in muscle L* or b* for either flock. In flock 1 however, muscle a* was higher for LL than SH ($P < 0.001$) and LB ($P < 0.01$) (Table 2-4) and in flock 2, muscle a* was higher for LL than SH ($P < 0.01$) (Table 2-5).

Housing treatment differences were not observed for individual strains for L* or b* in either flock (Tables 2-4 & 2-5). In both flock 1 and 2 however, SH muscle a* was higher in CC than in FC ($P < 0.05$) (Tables 2-4 & 2-5). No strain differences were observed within a treatment for L* or b* in either flock (Tables 2-4 & 2-5). However, within CC, flock 1 muscle a* was higher for LL than both SH ($P < 0.01$) and LB ($P < 0.001$), and within FC, flock 1 muscle a* was higher for LL than SH ($P < 0.05$) (Table 2-4). In flock 2, muscle a* was higher for CC LL than CC SH ($P < 0.05$) and was higher for both FC LL and FC LB than FC SH ($P < 0.05$) (Table 2-5).

2.3.5 Muscle Shear Force (SF)

For both flocks, when strains were combined, muscle SF did not differ between housing systems (Tables 2-4 & 2-5). When treatments were combined, muscle SF was higher for SH than LB in both flocks ($P < 0.05$) (Tables 2-4 & 2-5). No housing treatment differences were observed for individual strains, however strain differences were observed within CC for both flocks. Within CC, flock 1 muscle SF was higher for SH than both LL ($P < 0.05$) and LB ($P <$

0.01) (Table 2-4). In flock 2, muscle SF was higher for CC SH and CC LL than CC LB ($P < 0.05$) (Table 2-5).

2.3.6 Correlations

2.3.6.1 *pH, Colour and Shear Force*

When all treatments and strains were combined, a positive correlation was observed between muscle pH_u and L* (r = 0.15, $P = 0.02$), and a negative correlation was observed between ΔpH and L* (r = -0.16, $P = 0.01$) in flock 1 (Table 2-6). In flock 2, a positive correlation was observed between pH_{17} and b* (r = 0.17, $P = 0.006$) (Table 2-7), and negative correlations were observed between pH_{17} and SF (r = -0.13, $P = 0.04$) and between ΔpH and SF (r = -0.14, $P = 0.03$) (Table 2-9).

When all strains were combined, correlations were observed within each housing treatment. Within CC, positive correlations were found between pH_u and L* (r = 0.21, $P = 0.02$) and between muscle pH_{17} and a* (r = 0.19, $P = 0.03$), while negative correlations were found between muscle ΔpH and L* (r = -0.25, $P = 0.006$), ΔpH and b* (r = -0.19, $P = 0.04$) (Table 2-6) and between a* and SF (r = -0.18, $P = 0.05$) (Table 2-8) in flock 1. Within flock 2 CC, there was a positive correlation between muscle pH_u and a* (r = 0.19, $P = 0.03$) and within FC, there was a positive correlation between muscle pH_{17} and b* (r = 0.18, $P = 0.04$) (Table 2-7).

Within a housing treatment, correlations were observed for individual strains. Within CC, there was a positive correlation between muscle L* and SF for flock 1 SH (r = 0.35, $P = 0.02$), and a negative correlation between muscle a* and SF for flock 1 LB (r = -0.38, $P = 0.02$) (Table 2-8). In flock 2 CC, there were positive correlations between muscle pH_{17} and b* for SH (r = 0.31, $P = 0.04$), and between muscle pH_{17} and L* (r = 0.32, $P = 0.04$) and between ΔpH and L*

(r = 0.31, P = 0.04) for LB (Table 2-7). Additionally in CC, negative correlations were observed between muscle pH_{17} and SF (r = -0.33, P = 0.03) and between ΔpH and SF (r = -0.33, P = 0.03) for LB (Table 2-9). Within FC, no correlations were observed for individual strains in flock 1. In flock 2, positive correlations were observed between muscle pH_{17} and b* (r = 0.41, P = 0.008) and between muscle ΔpH and b* (r = 0.32, P = 0.05) for SH (Table 2-7). Positive correlations were also observed between muscle ΔpH and L* (r = 0.36, P = 0.02) (Table 2-7) and between a* and SF (r = 0.31, P = 0.05) for LB (Table 2-9). Negative correlations were observed between muscle pH_u and SF (r = -0.33, P = 0.04) for LL, and between muscle pH_u and L* (r = -0.37, P = 0.02), pH_{17} and SF (r = -0.35, P = 0.03), and between ΔpH and SF (r = -0.39, P = 0.01) for LB (Table 2-9).

2.3.6.2 *Shear Force and Bone Data*

When all treatments and strains were combined, a negative correlation was observed between BBS and SF (r = -0.18, P = 0.005) in flock 1 (Table 2-10). No correlations were observed between proportion of broken bones, pre- or post-slaughter, and SF for either flock, or between BBS and SF for flock 2. When strains were combined, a negative correlation was observed between BBS and SF in both flock 1 and 2 CC (r = -0.26, P = 0.003, and r = -0.25, P = 0.004, respectively) (Table 2-10 & 2-11). Within each housing treatment, correlations were observed for individual strains. In flock 1, there was a positive correlation between BBS and SF for FC LL (r = 0.38, P = 0.02) (Table 2-10). In flock 2, there was a negative correlation between pre-slaughter proportion of broken bones and SF for CC LB (r = -0.31, P = 0.04) (Table 2-11).

2.4 Discussion

2.4.1 Body Weight

Differences in hen BW between housing systems may be attributed, in part, to differing sources of stress in CC and FC. Whereas hens in CC may experience stress associated with behavioural and physical restrictions, hens in FC may experience social stress from the larger group size. Keeling *et al*. (2003) showed reduced hen BW in intermediate group sizes compared to small and large group sizes, which they attributed to social intolerance. Stress leads to the release of glucocorticoids from the adrenal gland, increasing whole body metabolism and therefore increasing energy demand (Selye, 1950). An increase in energy demand thereby depletes muscle glycogen stores (Zhang *et al*., 2009) and stimulates protein catabolism, affecting BW (Lin *et al*., 2004).

Body weight differences between housing systems may be further attributed to differing hen activity levels. Past research has shown that organically-raised chickens have lower BW than conventionally-raised chickens due to the increased energy demand from locomotory behaviour (Castellini *et al*., 2002; Branciari *et al*., 2009). Whereas hens in CC may perform more stereotypic locomotory activity due to behavioural restriction (Yue & Duncan, 2003), hens in FC have greater opportunity to express highly motivated and natural locomotory behaviour which may lead to reduced overall BW in FC as compared to CC. The difference in activity between housing systems may also be contributing to the reduced BW for FC SH compared to CC SH in flock 1, and decreased BW for FC LB compared to CC LB in flock 2. It is also important to note that an outbreak of cannibalism occurred in flock 2 as the result of construction in a room adjacent to the FC system (Jendral, 2012). Cannibalism-related deaths accounted for 1.9% of total flock 2 FC mortalities, all of which occurred in LB hens, post construction onset (Jendral,

2012). The stress from this outbreak may have been a contributing factor to the lower BW of FC LB compared to CC LB in flock 2.

Strain differences in BW are consistent with breeder averages (Shaver White, Lohmann LSL-Lite, and Lohmann Brown Classic Management Guides). As expected, the medium hybrid LB had a higher BW than LL and SH strains, both of which are light hybrids.

2.4.2 Breast Muscle Depth

In the current study, hens had similar BD despite differences in BW. Since BW has been shown to be positively correlated with *Pectoralis major* muscle weight and with muscle yield in broiler chickens (Le Bihan-Duval *et al*., 1998), and since *Pectoralis major* muscle weight and yield have been shown to be positively correlated with BD in broiler chickens (Scheuermann *et al*., 2003; Gaya *et al*., 2006), hens in CC in the current study would be expected to have increased BD as compared to hens in FC. Similarly, LB hens would be expected to have higher overall BD than SH and LL hens. However, whereas broiler chickens are highly selected for muscle mass and yield (Le-Bihan-Duval *et al*., 1998), laying hens are highly selected for egg production (Poggenpoel *et al*., 1996). Therefore, BW may not provide an accurate prediction of BD in laying hens. This point is exemplified in flock 2 where SH hens had lower BW than LL and LB hens, but had greater BD.

2.4.3 Muscle pH

Post-mortem muscle pH decline occurs as the result of lactate production due to muscle glycogen breakdown (Bendall, 1973). Treatment differences in post-mortem muscle pH might therefore be expected if either the peri-mortem muscle glycogen content differs (Berri *et al*., 2007; Zhang *et al*., 2009) or the rate of muscle glycogen breakdown differs (Berri *et al*., 2001).

In the current study, the absence of housing treatment differences in muscle pH therefore suggest that hens either had similar peri-mortem glycogen levels and similar rates of glycogen breakdown, or that hens had differing peri-mortem glycogen levels, and either similar or differing rates of glycogen breakdown. The lack of overall differences in BD between treatments and strains might suggest similar glycogen content across muscle samples, which would imply similar rates of glycogen breakdown between treatments.

The absence of treatment differences in muscle pH may be further explained by the differing environmental stressors experienced in the two housing systems. Such chronic stressors have been shown to induce muscle glycogen depletion (Zhang *et al*., 2009; Zulkifli *et al*., 2012). Given that hens housed in CC are both limited in movement and in their ability to express natural highly motivated behaviours (Appleby *et al*., 2002) it is expected that CC hens would experience increased stress leading to glycogen depletion. However, hens in FC would experience social stress as a result of large group size (Keeling *et al*., 2003), also resulting in glycogen depletion. The presence of stress in both housing systems and resultant glycogen depletion might therefore lead to similar muscle pH between housing systems.

In flock 1, lower muscle pH_{17} for LB compared to SH and LL suggests either greater peri-mortem muscle glycogen content for LB and similar or differing glycogen breakdown rate, or an absence of strain differences in peri-mortem muscle glycogen content and a higher rate of muscle glycogen breakdown for LB. Lower muscle pH_u for LB compared to SH and LL support that differences in muscle pH_{17} were not the result of differing rates of glycogen depletion. Rather, it is likely that LB had greater peri-mortem muscle glycogen content than SH and LL. This is further supported by a greater change in muscle pH from 17 min to 24 hr post-mortem for LB as compared to SH. Glycogen content likely differed between strains due to differences in

stress response. Past studies have shown a higher stress response in White Leghorn strains than in brown strains (Albentosa *et al*., 2003; Fraisse & Cockrem, 2006). Additionally, SH hens have demonstrated a poorer stress coping response as compared to medium brown hybrids and other white hybrids, as evident from increased aggravation due to changes in diet, lighting, and cage density (Hughes & Duncan, 1972). Stress may have also contributed to the performance of stereotypic behaviours for LL and SH (Yue & Duncan, 2003) which would also have contributed to glycogen depletion and consequent higher muscle pH for SH and LL.

The strain differences noted for flock 1 muscle pH were not present in flock 2. The absence of flock 2 differences in muscle pH suggests that LB in this flock may have experienced stress-induced glycogen depletion similar to SH and LL. Notably, an outbreak of cannibalism occurred during flock 2 and had a detrimental effect on LB hens in particular, which would have caused increased stress for those hens (Jendral, 2012).

2.4.4 Muscle Colour

The absence of housing differences in muscle L* and b* for all strains combined, and in muscle a* for LL and LB hens, is consistent with the absence of housing differences in muscle pH. Muscle pH can affect the rate of myoglobin oxygenation and consequent muscle pigment formation (Faustman & Cassen, 1990), and strong correlations have been shown for muscle L* and pH (Berri *et al*., 2005; Fletcher, 1999; Barbut, 1993). Given that muscle pH has been shown to affect muscle colour (Barbut, 1993), it would also be expected from the pH data of the current study that muscle a* would not differ between CC SH and FC SH. However, muscle colour can be affected by factors other than pH. Increased total muscle pigment content and myoglobin content for example, results in higher muscle a*, but does not always result in higher muscle pH (Castellini *et al*., 2002; Boulianne & King, 1995).

Differences in muscle colour may also be influenced by genetics (Berri *et al*., 2005). Increased muscle a* for LL hens in both flocks could be the result of naturally redder muscles for LL than SH and LB hens. Similar redness levels for LL and LB hens in flock 2 are consistent with the stress experienced by flock 2 LB hens as a result of cannibalism. Stress-induced glycogen depletion has been shown to produce higher muscle pH and darker muscle colour (Zhang *et al*., 2009; Owens & Sams, 2000).

2.4.5 Muscle Shear Force

Muscle shear force is determined, in part, by muscle glycogen content. Muscle with high glycogen content for example, typically yields lower SF values than muscle with low glycogen content (Mellor *et al*., 1958). Stress-induced muscle glycogen depletion would therefore be expected to yield higher muscle SF. In the current study, the lack of treatment differences in muscle SF may be attributed in part to stress-induced muscle glycogen depletion occurring in both housing systems. As described above, in CC, hens experience stress in relation to restricted behavioural expression and movement (Appleby *et al*., 2002). In FC, where behavioural expression and physical movement are supported, hens nonetheless experience social stress as a result of the large group size (Keeling *et al*., 2003). The absence of housing differences in SF is supported by the overall lack of treatment differences in muscle pH and muscle colour.

The higher muscle SF for CC SH than CC LB in flock 1, and for CC SH and CC LL than CC LB in flock 2, is consistent with strain differences in stress response within CC. As discussed above, light hybrids have been shown to have a higher stress response than brown strains (Albentosa *et al*., 2003; Fraisse & Cockrem, 2006), and SH have shown a poorer stress coping response as compared to medium brown hybrids and other white hybrids (Hughes & Duncan, 1972). Stress response would affect muscle glycogen breakdown and consequent muscle SF

(Mellor *et al*., 1958). Notably however, this strain effect was not observed in flock 1 FC, despite similar muscle pH values for LB in both CC and FC. It is likely that factors other than muscle glycogen content are influencing muscle SF. Fanatico *et al*. (2007) for example, showed increased *Pectoralis major* muscle SF in broiler chickens raised with outdoor access as compared with conventionally-raised birds, which they attributed in part to the higher muscle protein content of the birds with outdoor access. In the current study, LB have greater BW than LL and SH, and might therefore also have greater breast yield (Le Bihan-Duval *et al*., 1998) and muscle protein content (Berri *et al*., 2001). The absence of strain differences in SF between CC LL and CC LB in flock 1 also supports that factors other than strain differences in stress response and resultant glycogen depletion are contributing to muscle tenderness. The absence of SF strain differences in FC of flock 2, despite strain differences in CC, may be further attributed to cannibalism related stress response of LB in that flock. Additional studies investigating muscle glycogen and protein content would further elucidate housing treatment and strain differences in muscle SF.

2.4.6 Correlations

Despite consistent treatment findings across muscle pH, colour and SF data, and despite established negative correlations between L* and pH (Barbut, 1993; Fletcher, 1999; Berri *et al*., 2005) and between SF and pH (Fletcher, 1999), few correlations were noted for these variables in the current study. Interestingly however, an overall negative correlation was noted between humeral BBS and muscle SF suggesting that, for hens with increased load-bearing activity and consequent structural bone preservation (Jendral *et al*., 2008), muscle glycogen content is high. In other words, hens who have opportunity to express load-bearing activity have decreased

muscle SF, which may be attributed to reduced stress and consequent glycogen depletion (Mellor *et al*., 1958).

2.5 Conclusion

Contrary to our hypothesis that hens in FC, who have greater opportunity to express highly motivated and locomotory behaviour, will exhibit improved meat quality over conventionally-caged hens, despite the social stress resulting from group size in FC, we did not find housing treatment differences in meat quality. Rather the findings of this research suggest that the environmental stressors present in both housing systems, similarly affect meat quality. Strain differences in muscle pH, a* and SF further indicate increased stress experienced by SH and LL hens. Notably, the absence of strain differences in flock 2 are consistent with the outbreak of cannibalism that occurred in this flock and had the greatest impact on LB hens. Additional studies investigating the physicochemical basis for variations in pH, glycogen and protein content and fibre types in the *Pectoralis major* muscle are recommended to further elucidate the effects of housing system and strain differences on hen stress response and consequent meat quality.

Table 2-1. Flock 1 and 2 80-week body weight and *Pectoralis major* muscle depth[1] of Shaver White (SH), Lohmann Lite (LL), and Lohmann Brown (LB) housed in conventional cages (CC) or furnished cages (FC).

Variables[2]	Housing Treatment	Strains Combined	SH	LL	LB
FLOCK 1					
BW80	Treatments Combined	1802.58 ± 13.95	1610.92^{c} ± 24.29	1725.22^{b} ± 24.42	2067.17^{a} ± 25.02
	CC	1830.93 ± 19.83	1664.14^{b} ± 33.52	1725.53^{b} ± 33.91	2103.13^{a} ± 35.60
	FC	1771.27 ± 20.30	1557.71^{c} ± 35.15	1724.91^{b} ± 35.15	2031.20^{a} ± 35.15
	Housing Probabilities	0.0366	0.0294	0.9898	0.1519
BD	Treatments Combined	—	17.38 ± 0.62	17.08 ± 0.60	17.08 ± 0.64
	CC	17.36 ± 0.41	18.07 ± 0.71	17.27 ± 0.71	16.75 ± 0.75
	FC	17.00 ± 0.56	16.68 ± 0.99	16.90 ± 0.97	17.40 ± 0.99
	Housing Probabilities	0.6035	0.2570	0.7653	0.5908
FLOCK 2					
BW80	Treatments Combined	1844.24 ± 13.85	1690.14^{c} ± 20.18	1755.37^{b} ± 20.07	2082.52^{a} ± 20.18
	CC	1888.73 ± 16.04	1696.85^{c} ± 27.90	1793.06^{b} ± 27.58	2176.29^{a} ± 27.90
	FC	1796.62 ± 16.85	1683.43^{b} ± 29.18	1717.69^{b} ± 29.18	1988.75^{a} ± 29.18
	Housing Probabilities	0.0004	0.7417	0.0702	< 0.0001
BD	Treatments Combined	—	16.39^{a} ± 0.33	14.85^{b} ± 0.31	15.10^{b} ± 0.35
	CC	15.34 ± 0.25	16.41^{a} ± 0.45	14.84^{b} ± 0.43	14.76^{b} ± 0.47
	FC	15.55 ± 0.26	16.37^{a} ± 0.46	14.85^{b} ± 0.45	15.44^{ab} ± 0.45
	Housing Probabilities	0.5536	0.9517	0.9934	0.2766

[1] Least square means ± standard error.
[2] BW80 = Body weight at 80 weeks (g); BD = Breast muscle depth (mm)
[a-c] Least square means with no common superscript are significantly different ($P \leq 0.05$) within a row.

Table 2-2. Flock 1 *Pectoralis major* muscle pH[1] of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages).

Variables[2]	Housing Treatment	Strains Combined	SH	LL	LB
pH_{17}	Treatments Combined	—	$6.64^{a} \pm 0.01$	$6.67^{a} \pm 0.01$	$6.59^{b} \pm 0.01$
	CC	6.64 ± 0.01	$6.64^{a} \pm 0.01$	$6.67^{a} \pm 0.01$	$6.60^{b} \pm 0.02$
	FC	6.63 ± 0.01	$6.64^{a} \pm 0.02$	$6.67^{a} \pm 0.02$	$6.58^{b} \pm 0.02$
	Housing Probabilities	0.3812	0.7124	0.6503	0.4884
pH_u	Treatments Combined	—	$5.86^{a} \pm 0.01$	$5.82^{a} \pm 0.01$	$5.75^{b} \pm 0.01$
	CC	5.82 ± 0.01	$5.86^{a} \pm 0.02$	$5.84^{a} \pm 0.02$	$5.76^{b} \pm 0.02$
	FC	5.80 ± 0.01	$5.85^{a} \pm 0.02$	$5.81^{a} \pm 0.02$	$5.73^{b} \pm 0.02$
	Housing Probabilities	0.1800	0.7830	0.2796	0.3330
Δ pH	Treatments Combined	—	$0.79^{b} \pm 0.02$	$0.85^{a} \pm 0.02$	$0.84^{a} \pm 0.02$
	CC	0.82 ± 0.01	0.79 ± 0.02	0.84 ± 0.02	0.84 ± 0.03
	FC	0.83 ± 0.01	$0.79^{b} \pm 0.03$	$0.86^{a} \pm 0.03$	$0.85^{ab} \pm 0.03$
	Housing Probabilities	0.5744	0.9999	0.5439	0.7128

[1] Least square means ± standard error.
[2] pH_{17} = pH measured at 17 min post-mortem; pH_u = pH measured at 24 h post-mortem; Δ pH = pH change from 17 min post-mortem to 24 h post-mortem.
[a-c] Least square means with no common superscript are significantly different ($P \leq 0.05$) within a row.

Table 2-3. Flock 2 *Pectoralis major* muscle pH[1] of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages).

Variables[2]	Housing Treatment	Strains Combined	SH	LL	LB
pH_{17}	Treatments Combined	—	6.58 ± 0.05	6.49 ± 0.05	6.52 ± 0.05
	CC	6.55 ± 0.03	6.61 ± 0.06	6.49 ± 0.06	6.54 ± 0.06
	FC	6.51 ± 0.04	6.54 ± 0.07	6.49 ± 0.07	6.51 ± 0.07
	Housing Probabilities	0.5168	0.4092	0.9559	0.7179
pH_u	Treatments Combined	—	5.85^{a} ± 0.02	5.86^{a} ± 0.03	5.77^{b} ± 0.02
	CC	5.81 ± 0.02	5.80 ± 0.03	5.85 ± 0.03	5.77 ± 0.03
	FC	5.85 ± 0.02	5.91^{a} ± 0.04	5.87^{ab} ± 0.04	5.78^{b} ± 0.04
	Housing Probabilities	0.1324	0.0498	0.6355	0.8776
Δ pH	Treatments Combined	—	0.72 ± 0.06	0.63 ± 0.06	0.75 ± 0.06
	CC	0.74 ± 0.04	0.81 ± 0.07	0.64 ± 0.08	0.77 ± 0.07
	FC	0.66 ± 0.05	0.63 ± 0.09	0.62 ± 0.09	0.73 ± 0.09
	Housing Probabilities	0.2296	0.1264	0.8408	0.7219

[1] Least square means ± standard error.
[2] pH_{17} = pH measured at 17 min post-mortem; pH_u = pH measured at 24 h post-mortem; Δ pH = pH change from 17 min post-mortem to 24 h post-mortem.
[a-c] Least square means with no common superscript are significantly different ($P \leq 0.05$) within a row.

Table 2-4. Flock 1 *Pectoralis major* muscle colour and shear force[1] of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages).

Variables[2]	Housing Treatment	Strains Combined	SH	LL	LB
L*	Treatments Combined	—	56.61 ± 0.63	55.45 ± 0.63	56.56 ± 0.64
	CC	56.27 ± 0.44	56.85 ± 0.75	55.65 ± 0.75	56.32 ± 0.77
	FC	56.15 ± 0.58	56.38 ± 1.01	55.26 ± 1.01	56.81 ± 1.01
	Housing Probabilities	0.8684	0.7146	0.7597	0.7023
a*	Treatments Combined	—	5.03^{b} ± 0.24	6.34^{a} ± 0.24	5.22^{b} ± 0.25
	CC	5.82 ± 0.17	5.63^{b} ± 0.29	6.77^{a} ± 0.29	5.06^{b} ± 0.30
	FC	5.25 ± 0.23	4.44^{b} ± 0.39	5.92^{a} ± 0.39	5.38^{ab} ± 0.39
	Housing Probabilities	0.0516	0.0213	0.0933	0.5250
b*	Treatments Combined	—	18.56 ± 0.25	18.53 ± 0.25	18.60 ± 0.25
	CC	18.61 ± 0.18	18.63 ± 0.31	18.69 ± 0.31	18.53 ± 0.33
	FC	18.51 ± 0.22	18.49 ± 0.38	18.37 ± 0.38	18.67 ± 0.38
	Housing Probabilities	0.7146	0.7785	0.5255	0.7809
SF	Treatments Combined	—	12.48^{a} ± 0.33	11.69^{ab} ± 0.33	11.22^{b} ± 0.34
	CC	12.04 ± 0.24	13.10^{a} ± 0.41	11.85^{b} ± 0.41	11.18^{b} ± 0.43
	FC	11.55 ± 0.30	11.85 ± 0.53	11.54 ± 0.52	11.26 ± 0.53
	Housing Probabilities	0.2153	0.0736	0.6472	0.9005

[1] Least square means ± standard error.
[2] L* = lightness; a* = redness; b* = yellowness; SF = Shear force (N).
[a-c] Least square means with no common superscript are significantly different ($P \leq 0.05$) within a row.

Table 2-5. Flock 2 *Pectoralis major* muscle colour and shear force[1] of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages).

Variables[2]	Housing Treatment	Strains Combined	SH	LL	LB
L*	Treatments Combined	—	50.57 ± 0.42	49.93 ± 0.42	49.55 ± 0.41
	CC	49.62 ± 0.33	50.35 ± 0.58	49.39 ± 0.58	49.11 ± 0.55
	FC	50.42 ± 0.35	50.79 ± 0.60	50.47 ± 0.60	50.00 ± 0.61
	Housing Probabilities	0.1078	0.6002	0.2132	0.2876
a*	Treatments Combined	—	2.74^{b} ± 0.28	4.03^{a} ± 0.28	3.43^{ab} ± 0.28
	CC	3.80 ± 0.21	3.39^{b} ± 0.38	4.48^{a} ± 0.38	3.52^{ab} ± 0.36
	FC	3.01 ± 0.24	2.09^{b} ± 0.41	3.58^{a} ± 0.42	3.35^{a} ± 0.42
	Housing Probabilities	0.0206	0.0280	0.1207	0.7540
b*	Treatments Combined	—	13.79 ± 0.24	13.33 ± 0.24	13.49 ± 0.23
	CC	13.52 ± 0.19	13.92 ± 0.33	13.39 ± 0.33	13.25 ± 0.31
	FC	13.54 ± 0.20	13.65 ± 0.34	13.27 ± 0.34	13.72 ± 0.34
	Housing Probabilities	0.9406	0.5586	0.7895	0.3202
SF	Treatments Combined	—	24.03^{a} ± 0.53	23.08^{ab} ± 0.53	21.88^{b} ± 0.51
	CC	22.63 ± 0.42	23.61^{a} ± 0.77	23.20^{a} ± 0.75	21.08^{b} ± 0.71
	FC	23.36 ± 0.42	24.45 ± 0.72	22.96 ± 0.73	22.67 ± 0.73
	Housing Probabilities	0.2321	0.4306	0.8165	0.1270

[1] Least square means ± standard error.
[2] L* = lightness; a* = redness; b* = yellowness; SF = Shear force (N).
[a-c] Least square means with no common superscript are significantly different ($P \leq 0.05$) within a row.

Table 2-6. Pearson correlation coefficients for Flock 1 muscle pH (pH_{17}, pH_u, ΔpH) and colour (L*, a*, b*) variables.

	pH_{17} vs pH_u	pH17 vs L*	pHu vs L*	Δ pH vs L*	pH17 vs a*	pHu vs a*	Δ pH vs a*	pH17 vs b*	pHu vs b*	Δ pH vs b*
CC LB	-0.405ǂ	-0.111	0.128	-0.143	-0.073	0.040	-0.067	-0.185	0.085	-0.159
FC LB	0.098	-0.053	0.202	-0.191	-0.079	-0.083	0.004	0.068	0.052	0.011
CC LL	0.052	-0.140	0.223	-0.270	0.041	-0.051	0.067	-0.188	0.177	-0.258
FC LL	0.371ǂ	0.127	0.094	-0.010	0.042	0.037	-0.011	0.020	0.002	0.026
CC SH	-0.143	-0.151	0.268	-0.280	0.172	-0.046	0.141	-0.048	0.156	-0.137
FC SH	0.081	0.157	0.138	-0.031	-0.096	0.082	-0.125	0.150	-0.135	0.203
CC	-0.053	-0.144	0.207ǂ	-0.245ǂ	0.194ǂ	0.080	0.059	-0.121	0.148	-0.186ǂ
FC	0.240ǂ	0.038	0.115	-0.082	-0.011	-0.031	0.025	0.059	-0.046	0.084
Overall	0.115	-0.042	0.153ǂ	-0.156ǂ	0.099	0.034	0.035	-0.032	0.049	-0.061

ǂ $P \leq 0.05$

Table 2-7. Pearson correlation coefficients for Flock 2 muscle pH (pH_{17}, pH_u, ΔpH) and colour (L*, a*, b*) variables.

	pH_{17} vs pH_u	pH17 vs L*	pHu vs L*	Δ pH vs L*	pH17 vs a*	pHu vs a*	Δ pH vs a*	pH17 vs b*	pHu vs b*	Δ pH vs b*
CC LB	-0.240	0.320ǂ	-0.153	0.309ǂ	0.031	0.253	-0.122	0.149	-0.135	0.181
FC LB	-0.139	0.216	-0.372ǂ	0.364ǂ	-0.221	0.227	-0.291	0.051	0.191	-0.060
CC LL	-0.163	-0.067	0.023	-0.081	-0.127	-0.142	-0.077	0.007	-0.112	0.033
FC LL	0.256	0.107	0.212	-0.040	-0.139	-0.201	0.001	0.064	0.018	-0.010
CC SH	0.025	-0.018	0.122	-0.081	0.195	0.099	0.114	0.308ǂ	0.163	0.176
FC SH	0.080	0.089	0.055	0.059	0.131	0.115	0.068	0.414ǂ	0.144	0.319ǂ
CC	-0.143	0.127	-0.005	0.090	-0.075	0.187ǂ	-0.162	0.148	-0.012	0.105
FC	0.075	0.146	0.020	0.112	-0.006	-0.070	0.031	0.183*	0.080	0.097
Overall	-0.058	0.123	0.022	0.080	-0.021	0.027	-0.032	0.173ǂ	0.011	0.122

ǂ $P \leq 0.05$

Table 2-8. Pearson correlation coefficients for Flock 1 muscle shear force (SF) and muscle pH (pH_{17}, pH_u, ΔpH) and colour variables (L*, a*, b*).

	pH17 vs shear force	pHu vs shear force	Δ pH vs shear force	L* vs shear force	a* vs shear force	b* vs shear force
CC LB	-0.021	-0.276	0.158	-0.116	-0.383ǂ	-0.315
FC LB	-0.098	-0.115	0.015	-0.087	0.145	-0.017
CC LL	0.036	-0.084	0.092	-0.224	0.050	0.049
FC LL	0.179	0.045	0.069	0.261	-0.091	0.057
CC SH	-0.019	0.008	-0.018	0.345ǂ	0.059	-0.064
FC SH	0.247	0.306	-0.126	0.270	-0.074	0.151
CC	0.037	0.003	0.021	0.037	-0.176ǂ	-0.058
FC	0.084	0.072	-0.012	0.138	-0.020	0.056
Overall	0.068	0.048	0.003	0.084	-0.073	-0.007

ǂ $P \leq 0.05$

Table 2-9. Pearson correlation coefficients for Flock 2 muscle shear force (SF) and muscle pH (pH_{17}, pH_u, ΔpH) and colour variables (L*, a*, b*).

	pH17 vs shear force	pHu vs shear force	Δ pH vs shear force	L* vs shear force	a* vs shear force	b* vs shear force
CC LB	-0.325ǂ	0.175	-0.326ǂ	-0.170	-0.182	-0.193
FC LB	-0.346ǂ	0.218	-0.385ǂ	-0.002	0.312ǂ	0.140
CC LL	-0.068	0.076	-0.081	-0.186	0.182	0.006
FC LL	-0.208	-0.332ǂ	0.030	0.034	0.193	0.141
CC SH	-0.204	-0.135	-0.102	0.034	-0.029	-0.213
FC SH	-0.006	-0.173	0.035	0.030	0.266	0.222
CC	-0.121	0.074	-0.129	-0.044	-0.007	-0.075
FC	-0.129	-0.024	-0.115	0.047	0.090	0.177
Overall	-0.132ǂ	0.043	-0.138ǂ	0.006	0.017	0.022

ǂ $P \leq 0.05$

Table 2-10. Pearson correlation coefficients for Flock 1 muscle shear force (SF) and bone data.

	BBS vs SF	Pre-slaughter palpation (proportion of bone fractures) vs SF	Post-slaughter palpation (proportion of bone fractures) vs SF
CC LB	-0.123	0.124	0.059
FC LB	-0.010	-0.176	-0.218
CC LL	-0.247	0.219	-0.132
FC LL	0.380ǂ	-0.126	-0.184
CC SH	-0.119	-0.016	-0.067
FC SH	0.266	0.026	0.293
CC	-0.261ǂ	0.016	0.016
FC	0.043	-0.086	-0.009
Overall	-0.178ǂ	-0.026	0.027

ǂ $P \leq 0.05$

Table 2-11. Pearson correlation coefficients for Flock 2 muscle shear force (SF) and bone data.

	BBS vs SF	Pre-slaughter palpation (proportion of bone fractures) vs SF	Post-slaughter palpation (proportion of bone fractures) vs SF
CC LB	-0.098	-0.310ǂ	-0.019
FC LB	0.200	-0.170	-0.008
CC LL	-0.160	0.054	0.021
FC LL	0.206	-0.031	-0.016
CC SH	0.111	0.267	-0.006
FC SH	0.132	-0.098	-0.092
CC	-0.254ǂ	0.019	-0.016
FC	0.021	-0.002	0.060
Overall	-0.073	0.007	0.013

ǂ $P \leq 0.05$

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CHAPTER 3. Physiochemical determinants of pH in *Pectoralis major* of three strains of laying hens housed in conventional and furnished cages

3.1 Introduction

Muscle pH is commonly measured to assess meat quality because it influences visual and sensory characteristics such as color (Ahn & Maurer, 1990), tenderness (Jeleníková *et al*., 2008) and water-holding capacity (WHC) (Hughes *et al*., 2014). Meat pH that is too high is typically associated with darker hues, decreased tenderness and increased WHC (Ahn & Maurer, 1990; Barbut *et al*., 2005; Berri *et al*., 2007; Jeleníková *et al*., 2008), whereas excessive post-mortem pH decline has been associated with protein denaturation, paleness, extreme tenderness and lower WHC (Ahn & Maurer, 1990; Barbut *et al*., 2005; Berri *et al*., 2005; Le Bihan-Duval *et al*., 2008). Traditionally, the post-mortem decline in muscle pH has been attributed to glycogenolysis resulting in lactate accumulation (Bendall, 1973; Berri *et al*., 2007; Wang *et al*., 2013; England *et al*., 2014; England *et al*., 2016). However, lactate accumulation only accounts for a portion of the post-mortem pH decline (England *et al*., 2016), and significant dissociations have been noted between glycolytic potential and pH (Monin & Sellier, 1985), between post-mortem glycogenolysis and lactate accumulation (England *et al*., 2016; Matarneh *et al*., 2015), and between lactate accumulation and pH (Matarneh *et al*., 2015; Monin & Sellier, 1985). The underlying physicochemical basis for these dissociations has not been resolved to date.

Within biological systems, pH has been shown to be controlled by complex physicochemical relationships encapsulated in the Stewart model of acid-base regulation (Stewart, 1981; Stewart, 1983) that has been validated across several species (Putman *et al*., 2003; Stämpfli & Constable, 2003; Lindinger *et al*., 2005; Stämpfli *et al*., 2006; Lindinger & Heigenhauser, 2011). This model of acid-base regulation stipulates that a change in the

dependent variable, $[H^+]$, is determined by the balance struck by three systems-independent variables acting within the constraints of physicochemical laws of electrical neutrality, conservation of mass, and mass action equilibrium constants (*i.e.*, dissociation of weak acids; formation of bicarbonate ion; dissociation of water; formation of carbonate ion) (Stewart, 1981; Stewart, 1983). The first system-independent variable, the strong ion difference ([SID]), is defined as the sum of fully dissociated strong base cations (*i.e.*, $[Ca^{2+}] + [Mg^{2+}] + [Na^+] + [K^+]$) minus the sum of strong acid anions (i.e., $[Cl^-] + [Lac^-]$), and has been shown to vary inversely with muscle $[H^+]$ (Stewart, 1981; Stewart, 1983). The second variable is the net concentration of partially dissociated nonvolatile acids including metabolites, phosphates and soluble proteins termed $[A_{tot}]$. Such weak acids (HA) partially dissociate and therefore $[H^+]$ varies in proportion to increases in muscle $[A_{tot}]$ (Stewart, 1983). The third variable is the partial pressure of CO_2 (PCO_2), which exerts its acid properties when hydrated to form carbonic acid (H_2CO_3) and ultimately dissociated to bicarbonate (HCO_3^-) + H^+ (Stewart, 1981; Stewart, 1983).

A fundamental tenet of the Stewart model of acid-base regulation is that variation in $[H^+]$ between neighboring compartments does not result from the physical movement of H^+ across membranes. Rather, $[H^+]$ is determined by the net transmembrane movement of strong base cations, strong base anions, weak acids, CO_2 and the instantaneous establishment of new equilibria between the three systems-independent variables (Stewart, 1983). Although the Stewart model of acid-base regulation provides the essential conceptual and empirical framework to investigate variations in post-mortem acidification of muscle, it has not been applied to meat science.

In previous studies, *pectoralis major* muscle pH has been shown to differ between chicken strains (Berri *et al*., 2005; Fanatico *et al*., 2007) and between different housing

environments (Castellini *et al*., 2002; Fanatico *et al*., 2007). Additionally, in the research presented in Chapter 2 in which meat quality in 80 to 81 week old laying hens was evaluated, it was reported that *pectoralis major* pH at 17 min post-mortem (pH_{17}) differed between three strains of laying hens housed in an established production setting that included both conventional cage (CC) and furnished cage (FC) housing systems (Jendral, 2012). The purpose of the present study was to investigate the three systems-independent variables in post-mortem *pectoralis major* muscles of flock 1 birds from the previous study (Chapter 2) in order to understand the absence of variations in muscle pH between housing systems and to further evaluate the variations in muscle pH between genetic strains. Furthermore, the purpose of this study was to evaluate the Stewart Model by comparing measured values of $[H^+]$ with values calculated using equations derived by Stewart (Stewart, 1981; Stewart, 1983). It was hypothesized that the three systems-independent variables would be susceptible to ante-mortem and post-mortem changes imposed by production conditions that can account for variations in meat $[H^+]$ between bird strains and between housing environments.

3.2 Materials and methods

3.2.1 Animals and experimental design

Experiments were conducted in accordance with the guidelines of the Canadian Council for Animal Care and were authorized by the Animal Care and Use Committee of the Faculty of Agriculture at Dalhousie University (formerly Nova Scotia Agricultural College - NSAC) and by the Animal Care and Use Committee for Livestock at the University of Alberta. A flock comprised of 280 Shaver White (SH), 280 Lohmann Lite (LL), and 280 Lohmann Brown (LB) laying hens was housed at the Atlantic Poultry Research Centre (APRC) of the NSAC. Each hen strain represented a single genetic line. Upon arrival, 19 week old birds (Clarke's Chick

Hatchery, Burtt's Corner, New Brunswick) were randomly assigned to either a conventional cage (CC) or furnished cage (FC). Ambient temperature was maintained at 23°C and lighting was gradually increased from 11.5 to 15 hours per day by 25 weeks of age. Birds received a standard layer diet (National Research Council (NRC), 1994) and fresh water *ad libitum*.

The CC system was comprised of nine mobile batteries each containing two tiers of four cages for a total of 72 conventional cages (Jendral *et al*., 2012). Twenty-four cages were allocated to each bird strain. Each CC (60 cm wide × 55 cm deep × 45 cm high) housed 5 hens of the same strain providing 660 cm^2 of floor space per hen.

The FC system was comprised of 12 furnished battery cages (240 cm wide × 110 cm deep × 50 cm high) (Jendral *et al.*, 2012). Four cages were allocated to each strain and each FC housed forty birds, providing 660 cm^2 of floor space per hen. Each FC also contained a nest box, perches, and a dust bath. Nest boxes contained an artificial turf lining and measured 60 cm wide × 55 cm deep, which provided an additional 92 cm^2 of space per hen. Three semi-circular hardwood perches measuring 5 cm wide × 2.5 cm deep spanned the length of the cage. An elevated metal dust bath measuring 60 cm wide × 20 cm deep and containing a substrate made of wood shavings and feed was opened daily 8 hours after the beginning of the daylight cycle and was closed 45 min before the end of the daylight cycle in order to prevent dust bath egg-laying.

3.2.2 Processing and tissue collection

All hens were processed between 80 and 81 weeks of age. In the FC system, twenty five hens were randomly selected for processing from each of the 12 FC and in the CC system, for each of the nine batteries, all hens from one randomly selected cage per strain (*i.e., three CC per battery*) were designated for processing. Hens were gently removed from cages, weighed and scored for feather condition, palpated for bone fractures (Jendral *et al.*, 2012) and transported to

the processing facility at the APRC. Since muscle samples were collected to assess the effect of genetic strain and housing environment on meat quality, as determined in part by muscle pH, it was necessary to minimize the impact of other factors that also affect meat quality. Therefore, to minimize stress associated with transportation and feed restriction, hens received feed and water *ad libitum* up to the time of transport, and lights were dimmed during handling. Further, transportation crates were lined with bedding to prevent injury, meal worms were scattered on the floor of the crates, and crate stocking density was limited to 5 hens per crate.

Hens were gently and individually lifted from their transportation crates at processing. To minimize the time between shackling and stunning and to further minimize stress, only five hens were processed at a time. Hens were quickly rendered unconscious by an electrical stun and the right jugular artery was immediately severed. Hens were bled out for 90 s, placed in a scalder at 57°C for two minutes and then in a feather plucker for 45 sec. Following this process, hens were re-palpated for post-processing bone fractures (Jendral *et al.*, 2012). At 17 minutes post-mortem, 2.5 cm^3 samples were extracted from the right superior *pectoralis major* muscle (temperature: 17 ^oC) and snap frozen in liquid nitrogen. Muscle samples were subsequently transported on dry ice to the University of Alberta and stored at –80°C until further analysis. To account for variations in muscle size, average muscle depth of the left *pectoralis major* was determined.

3.2.3 Muscle analysis

3.2.3.1 *Muscle sample preparation, wet-to-dry weight ratio and total tissue water (TTW)*

Approximately 600 mg of each muscle sample was freeze-dried, dissected free of blood and connective tissue, and stored (–80°C) for later analysis of strong base cations and strong acid anions. The remaining snap frozen portion of each sample was also stored at –80°C for later

analysis. A portion of each sample (450-500 mg) was weighed before and after lyophilisation and used to calculate the wet-to-dry weight ratio and TTW.

3.2.3.2 *Glycogen, lactate and glycolytic potential*

Muscle glycogen content was determined spectrophotometrically on snap frozen samples using the phenol-sulfuric acid method (Lo *et al.*, 1970; Morifuji *et al.*, 2005). Approximately 20-40 mg of freeze-dried muscle tissue was immersed in 0.85 mL of ice cold 30% KOH saturated with sodium sulphate. Sample tubes were briefly centrifuged (IEC Centra-7R, International Equipment Company) at 1,000 rpm and then placed in a boiling water bath for 30 min. After cooling for 10 min, 1 mL of 95% ethanol was added to the sample tubes which were then cooled for 30 min and subsequently centrifuged at 840×g (2000 rpm) for 30 min. Supernatants were aspirated and 5 mL of MilliQ was added to the sample tubes to dissolve the glycogen precipitate. Muscle glycogen samples were transferred to cuvettes in duplicate 100 µL aliquots and 100 µL of 5% phenol was added to each cuvette. Samples were vortexed and 0.5 mL of 98% H_2SO_4 was added with the acid stream being directed against the sample surface for homogeneity. Sample cuvettes were then incubated at room temperature for 10 min, vortexed, and incubated for an additional 20 min at room temperature. Absorbance of samples were read at 490 nm using a spectrophotometer (Utrospec 3000, Amersham Pharmacia Biotech). Sample blanks of MilliQ water were prepared with the phenol and H_2SO_4 following the same method used for the samples.

Muscle lactate content was measured according to Putman *et al.* (1993; 1998). Approximately 100-150 mg of snap frozen muscle was pulverized in liquid nitrogen, then extracted in 6 volumes of ice-cold 6% (v/v) perchloric acid using a Polytron (Brinkmann Instruments, Mississauga, ON, Canada), and cleared by centrifugation (3,000×g) (IEC Micromax

RF). A reaction medium consisting of a glycine-hydrazine buffer, NAD and lactate dehydrogenase was added to sample PCA extracts in order to catalyze the reaction: Lac- + NAD → pyruvate + NADH. Lactate concentrations were assayed at 340 nm using a 96-well plate reader (BioTek EL808, BioTek Instruments). Each well contained 10 µL of sample, 10 µL of 6% PCA and 280 µL of reaction medium. Muscle lactate concentration ([lactate$^-$]) was calculated using TTW.

Glycolytic potential was calculated using a modified form of the equation published by Monin and Sellier (1985). The modified equation excluded glucose and glucose-6-phosphate, to reflect the fact that glycogen plus lactate account for 90% of the absolute glycolytic potential, as well as ~90% of the difference in glycolytic potential between experimental conditions (Monin & Sellier, 1985; Matarneh *et al.*, 2015; England *et al.*, 2016). Glycolytic potential was calculated as follows:

$$\text{glycolytic potential} = 2 \times (\text{glycogen}) + \text{lactate} \quad (1)$$

3.2.3.3 *Total muscle protein*

Total protein was measured using the method of Lowry *et al.* (1951). Approximately 100-150 mg of snap frozen muscle was homogenized in 9 volumes of MilliQ ultrapure water using a Polytron (Brinkmann Instruments). Duplicate 10 µL aliquots were diluted five-fold with MilliQ ultrapure water, mixed with 100 µL of 0.3 M KOH (Sigma-Aldrich, Oakville, ON, Canada) and incubated for 30 min at 37ºC. Duplicate 50 µL aliquots of the solubilized protein samples were then added to a clean disposable glass tubes and 2.5 mL of Lowry C solution was added. The composition for 100 mL of Lowry C solution was as follows: 61.7 mL 2% sodium carbonate, 35.7 mL MilliQ water, 1.3 mL 0.5% cupric sulfate, and 1.3 mL 1.0% sodium potassium tartrate. After a 10 min incubation at room temperature, 150 µL of Folin–Ciocalteu

reagent was added to each sample tube while mixing. Following a 45 min incubation, 300 µL was added to each well and protein concentration was assayed at 750 nm using a 96-well Absorbance Microplate Reader (BioTek EL808, BioTek Instruments, Vermont, USA).

3.2.3.4 *Measurement of muscle Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}*

Muscle samples were prepared according to Wang *et al.* (1996). This involved dissolving 20 mg of freeze dried muscle in 2 mL of 1N nitric acid (HNO_3) (TraceSELECT Ultra, Sigma-Aldrich) in a glass-covered 10 ml glass beaker, and incubating at 50°C for 48 hours for elemental extraction (Fisher Econotemp Laboratory Oven Model 15G). Samples were then filtered (Whatman no. 2 paper 110 mm, Fisher Scientific, Edmonton, AB, Canada), diluted in MilliQ ultrapure water and placed in polypropylene vials with aluminum free caps (Fisher Scientific). Samples were stored overnight at 4°C prior to analysis by inductively–coupled plasma–optical emission spectrometry (ICP–OES). To avoid exogenous elemental contamination, all glassware was acid–washed in 2% (V/V) HNO_3. Positive controls were prepared from freeze-dried hen fecal samples, while negative controls (field reagent blanks) contained only MilliQ ultrapure water. Additionally, laboratory reagent blanks consisting of pure HNO_3 were prepared following the same procedure used for muscle sample preparation. The contents of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} within positive controls were 101.00 ± 2.42 , 9.07 ± 0.15, 5.55 ± 0.10 and 46.54 ± 0.40 mg/g dry weight (dw), respectively.

Muscle contents of Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} were measured using ICP–OES (Optima ICP–OES 2100 DV, Perkin Elmer, Inc., Waltham, MA, USA) (Ashoka *et al.*, 2009; Soglia *et al.*, 2015); ICP–OES wavelengths were set to 317.077 nm, 279.077 nm, 589.592 nm and 766.490 nm for measurement of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}, respectively. Standard solutions were prepared in 2% (v/v) HNO_3 from commercial stock preparations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} (TraceSELECT,

Sigma-Aldrich). Sample aspiration and drain tubes were placed around the pump head and clipped into the tubing slots. The plasma flame was then ignited and the system was flushed with 4% (v/v) HNO_3 for twenty minutes, followed by a 2% (v/v) HNO_3 flush for ten minutes to minimize tube contamination. Sample analysis was carried out in the following order: laboratory reagent blanks, field reagent blanks, standards, positive controls, and finally muscle sample extracts. The aspiration tube was rinsed in MilliQ ultrapure water and dried between each sample aspiration. All measures were completed in triplicate. Muscle cation contents (mg/g dw) were converted to mEq/L of TTW. The concentration sum of strong base cations ($\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$) was calculated.

3.2.3.5 *Measurement of muscle Cl^-*

Muscle chloride content was determined by titration according to Cotlove (1963; 1968) with modifications (Wang *et al.*, 1996). Briefly, 50 mg of freeze-dried muscle samples were digested in 10 mL 0.1 N HNO_3 for 16 h at room temperature, cleared by centrifugation (1,500×g) (IEC Centra-7R, International Equipment Company, MA, USA), and the pH was adjusted to 7.5 (6.5-8.5) with NaOH (Sigma-Aldrich). The molar quantity of Cl^- was measured in duplicate 2 mL aliquots of the supernatant. Samples were transferred to 20 mL beakers containing 30 µL of 0.25 M K_2CrO_4 indicator (Fisher Scientific) and titrated using 0.1 M $AgNO_3$ (Fisher Scientific). Duplicate blank solutions consisted of only 0.1 N HNO_3. To avoid exogenous elemental contamination, all glassware was acid-washed in 2% (V/V) HNO_3. The concentration of Cl^- in extracts (moles/L) and muscle $lactate^-$ content (mmol/kg ww) were converted to mEq/L of TTW. The concentration sum of strong acid anions ($\sum[Cl^-]+[lactate^-]$) was calculated.

3.2.3.6 *Calculation of [SID]*

The [SID] was calculated using the following equation (Stewart, 1981; Stewart, 1983):

$$[SID] = ([Ca^{2+}] + [Mg^{2+}] + [Na^{+}] + [K^{+}]) - ([Cl^{-}] + [lactate^{-}]) \quad (2)$$

where all concentrations are expressed as mEq/L TTW.

3.2.3.7 *CO_2 content, partial pressure of CO_2 (PCO_2) and CO_2 solubility constant*

Muscle CO_2 was quantified for 7 randomly selected hens from each of the 6 groups (n = 42) according to the method of Pörtner *et al*. (1990) with modifications. Briefly, 100-200 mg of snap frozen muscle tissue was pulverized under liquid nitrogen, weighed, transferred to sealed Vacutainers (BD Vacutainer Blood Collection Tubes, Becton Dickinson Labware, Mississauga, ON) and extracted in 2 mL of 0.01 M HCl (Fisher Scientific). Using a Hamilton syringe, 200 µL of the sample headspace was injected into a Scion 456 gas chromatograph (GC) (Bruker Daltonics, East Milton, ON, Canada) equipped with Compass CDS software (Bruker Daltonics), an HP Plot Q sample column and a thermal conductivity detector set at 180 ºC. Samples were manually injected into the GC split programmed temperature vaporizing (PTV) injector at 55 ºC with a split ratio of 20:1. Helium was the carrier gas with a constant flow of 15 $mL \cdot min^{-1}$ and the oven was held at 35 ºC for the total sample run time of 2 min. Molar CO_2 content was determined against standards of CO_2 gas. Samples and standards were run in triplicate.

The CO_2 solubility coefficient (SCO_2) was calculated at 17ºC, which was the ambient temperature of the post-mortem muscle processing environment. Using previously reported SCO_2 coefficients at 0ºC (0.0895 $mmol \cdot L^{-1} \cdot mmHg^{-1}$) (Carroll *et al.*, 1991), 10ºC (0.0597 $mmol \cdot L^{-1} \cdot mmHg^{-1}$) (Harrison, 1988), 15ºC (0.0499 $mmol \cdot L^{-1} \cdot mmHg^{-1}$) (Truchot, 1976), 20ºC (0.0452 $mmol \cdot L^{-1} \cdot mmHg^{-1}$) (Harrison, 1988) and 37ºC (0.0308 $mmol \cdot L^{-1} \cdot mmHg^{-1}$) (Arthurs & Sudhakar, 2005), regression analysis was completed using SigmaPlot Version 12.5 (SYSTAT Software Inc., San Jose, CA) to derive SCO_2 at a temperature (T) of 17ºC. The resulting equation was as follows:

$$SCO_2 = 4x10^{-5} (T^2) - 0.0032 (T) + 0.0887, \quad R^2=0.995, P<0.0001 \qquad (3)$$

The derived value for SCO_2 at 17ºC was 0.04586 mmol·L^{-1} · $mmHg^{-1}$.

3.2.3.8 *Muscle bicarbonate ([HCO_3^-]), anion gap ([A^-]) and [Atot]*

Muscle [HCO_3^-] was calculated according to Stämpfli *et al.* (2006) using the following equation:

$$[HCO_3^-] = SCO_2 \times PCO_2 \times 10^{(pH - pK'_1)} \qquad (4)$$

where SCO_2 = 0.04586 Mol·L^{-1}·$mmHg^{-1}$ (eq. 3) and pK'_1 = 6.120.

The [A^-] was calculated according to Stämpfli *et al.* (2006) using the strong ion electroneutrality equation:

$$SID - [HCO_3^-] - [A^-] = 0 \qquad (5)$$

[A_{tot}] was then calculated also according to Stämpfli *et al.* (2006) .

$$[A_{tot}] = [A^-] + ([H^+]_{measured} \times [A^-] / K_A) \qquad (6)$$

where $K_A = 3.0 \times 10^{-7}$ Eq/L and [A^-] and $[H^+]_{measured}$ are expressed as Eq/L.

The contribution of muscle proteins to [A_{tot}] (*i.e.*, $[A_{tot}]_{protein}$) was calculated from total protein and TTW, and using a net charge of 2.45 per mg/dL, as previously reported (Putman *et al.*, 2003). The contribution of other organic non-volatile weak acid buffers to [A_{tot}] (*i.e.*, $[A_{tot}]_{weak\ acids}$) was calculated as the difference between [A_{tot}] and $[A_{tot}]_{protein}$ since [A_{tot}] represents the sum of $[A_{tot}]_{protein}$ and $[A_{tot}]_{weakacids}$.

3.2.3.9 *Measurement of muscle pH and conversion to [H^+]*

Muscle pH was measured using the homogenate method (Santé & Fernandez, 2000; Yu *et al.*, 2005; Wang *et al.*, 2013). Briefly, 0.5 g of snap frozen muscle tissue was homogenized in 5

mL of ice cold buffer containing 5 mM iodoacetate (Sigma-Aldrich) and 150 mM KCl (Sigma-Aldrich) using a Polytron homogenizer (Brinkmann Instruments). Iodoacetate was included in the buffer to arrest glycolytic activity (England *et al.*, 2014). After mixing in sealed tubes, pH was measured directly in ice cold homogenates using a calibrated Accumet Basic pH meter (Accumet Basic AB15, Fisher Scientific). Measured muscle pH was converted to $[H^+]$ using equation 7 and expressed as nEq/L.

$$[H^+] = 10^{-pH} \times 10^9 \tag{7}$$

3.2.3.10 *Calculation of $[H^+]$*

Muscle hydrogen ion concentration $[H^+]$ was calculated for 7 randomly selected hens from each of the 6 groups (n = 42) using the equation derived by Stewart (Stewart, 1981; Stewart, 1983; Jones, 1987). The following equation (eq. 8) defines the empirical relationship between the dependent variable $[H^+]$ and the three systems-independent variables, [SID], $[A_{tot}]$ and PCO_2, within the constraints of physicochemical laws of electrical neutrality, conservation of mass, and mass action equilibrium constants.

$$[H^+]^4 + (K_A + [SID])[H^+]^3 + \{K_A([SID] - [A_{tot}]) - (K_C PCO_2 + K'_W)\} \times [H^+]^2 - \{K_A(K_C PCO_2 + K'_W) + (K_3 K_C PCO_2)\}[H^+] - (K_A K_3 K_C PCO_2) = 0 \tag{8}$$

The mass action equilibrium constants were as follows: (i) dissociation of weak acids, $K_A = 3.0 \times 10^{-7}$ Eq/L; (ii) formation of bicarbonate ion, $K_C = 2.58 \times 10^{-11}$ $(Eq/L)^2$; (iii) dissociation of water, $K'_W = 4.4 \times 10^{-14}$ $(Eq/L)^2$; (iv) formation of carbonate ion, $K_3 = 6.0 \times 10^{-11}$ Eq/L. The corresponding pH values were also calculated (eq. 7).

3.2.4 Statistical analysis

Data are reported as least square means ± SEM. Response variables were analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, 2013) with *pectoralis major* muscle depth used as a covariate, day of slaughter used as a random effect and individual cage number used as the nested random effect. Statistical analyses were performed to assess treatment, strain and treatment by strain effects. Differences between group means were detected using the least significant differences test for planned comparisons. To examine the relationships between measured $[H^+]$ and calculated $[H^+]$, and between measured pH and calculated pH regression analyses were completed (SigmaPlot Version 12.5, SYSTAT Software Inc.). The level of significance for all analyses was assessed at $P \leq 0.05$. Statistical trends were noted when $0.05 < P \leq 0.1$. *P*-values are reported.

3.3 Results

3.3.1 *Pectoralis major* muscle depth, wet-to-dry weight ratio and total tissue water (TTW)

The average *pectoralis major* muscle depth for all groups was 17.02 ± 0.24 cm and did not differ between strains ($P > 0.11$) or between housing treatments ($P > 0.48$). The muscle wet-to-dry weight ratio did not differ between bird strains ($P > 0.80$) or between CC and FC systems ($P = 0.83$) (Table 3-1). Similarly, muscle TTW did not differ between bird strains ($P > 0.80$) or between CC and FC systems ($P = 0.81$) (Table 3-1).

3.3.2 Glycogen content, lactate content and glycolytic potential

Muscle glycogen content varied between 47.9 and 54.5 mmol/kg wet weight (ww) (Figure 3-1A) and fell within the range of previously reported data (Zhang *et al.*, 2009; Wang *et*

al., 2013). Independent of housing system, there was a significant strain effect in which glycogen content was elevated by 5.1 mmol/kg ww in SH hens ($P < 0.02$) compared with LL hens. Within the CC system, glycogen content was 6.6 mmol/kg ww higher in SH compared with LL hens ($P < 0.03$). Glycogen content did not differ between strains within the FC system ($P > 0.26$). Muscle glycogen content did not differ between the CC (51.4 ± 1.3) and FC (51.0 ± 1.3) housing systems ($P > 0.82$).

Muscle lactate content varied between 36.9 and 48.4 mmol/kg ww (Figure 3-1B) and was similar to previously reported values for post-mortem poultry muscles (Berri *et al*., 2007; Debut *et al*., 2005; Le Bihan-Duval *et al.*, 2008). Strain differences were noted for muscle lactate content that were independent of the housing system; muscle lactate was 8.3 and 9.1 mmol/kg ww greater in LB hens compared with SH ($P < 0.005$) and LL ($P < 0.003$), respectively. Within the CC housing system, muscle lactate content was 10 - 11.5 mmol/kg ww greater in LB compared with LL ($P < 0.005$) and SH ($P < 0.001$) hens, respectively (Figure 3-1B). Within the FC system, muscle lactate content trended 8.4 mmol/kg ww higher in LB ($P = 0.06$), compared with LL hens. Muscle lactate content did not differ between CC (41.3 ± 1.3) and FC (43.3 ± 1.5) housing systems ($P > 0.32$).

Glycolytic potential of hen *pectoralis major* varied between 134.5 and 152.6 mmol/kg ww (Figure 3-1C) and was similar to previously reported data (Debut *et al*., 2005; Le Bihan-Duval *et al.*, 2008). Strain differences were noted for glycolytic potential that were independent of the housing system; glycolytic potential was elevated by 11.0 mmol/kg ww in SH ($P < 0.02$) and by 16.8 mmol/kg ww in LB hens ($P < 0.001$), compared with LL hens. Within the CC system, the glycolytic potential of LB hens was elevated by 17.3 mmol/kg ww compared with LL hens ($P < 0.005$); it was also 11.5 mmol/kg ww higher in SH compared to LL hens ($P <$

0.05). Similarly, within the FC system the glycolytic potential of LB hens was 16.3 mmol/kg ww higher than LL hens ($P < 0.03$). Glycolytic potential did not differ between CC (144.1 ± 2.5) and FC (145.3 ± 2.6) housing systems ($P > 0.72$).

3.3.3 Strong base cations and strong acid anions

3.3.3.1 *Muscle concentrations of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}*

Muscle [Ca^{2+}] varied between 30.7 – 37.9 mEq/L (Figure 3-2A) and did not differ between bird strains ($P > 0.32$). Additionally, muscle [Ca^{2+}] did not differ between CC (33.2 ± 1.9) and FC (36.8 ± 2.7) housing systems ($P > 0.27$). Muscle [Ca^{2+}] values fell within the range of previously reported data (Al-Najdawi and Abdullah, 2002).

Muscle [Mg^{2+}] varied between 43.6 – 46.9 mEq/L (Figure 3-2B) and did not differ between strains ($P > 0.23$). In addition, muscle [Mg^{2+}] did not differ between CC (44.9 ± 0.9) and FC (45.7 ± 1.3) housing systems ($P > 0.61$). Muscle [Mg^{2+}] values were also comparable to previously reported data (Misra *et al*., 1980; Sun *et al*., 2011).

Muscle [Na^{+}] varied between 25.9 – 34.3 mEq/L (Figure 3-2C) and fell between the minimum and maximum values previously reported (Misra *et al*., 1980; Al-Najdawi and Abdullah, 2002). Independent of the housing system, muscle [Na^{+}] was 3.5 and 7.2 mEq/L greater in LL ($P < 0.05$) and LB hens ($P < 0.001$) compared with SH hens; [Na^{+}] was also 3.7 mEq/L higher in LB compared with LL ($P < 0.05$). In the CC system, [Na^{+}] was 4.3 and 6.6 mEq/L greater in LB hens compared with LL ($P < 0.04$) and SH ($P < 0.002$) hens, respectively. Within the FC system, muscle [Na^{+}] was similar for LL and LB hens ($P > 0.28$); [Na^{+}] was 7.7 mEq/L greater in LB ($P < 0.02$) compared to SH hens. Muscle [Na^{+}] did not differ between CC (28.9 ± 0.8) and FC (30.8 ± 1.1) housing systems ($P > 0.18$).

Muscle [K^+] varied between 110.9 – 120.7 mEq/L (Figure 3-2D) and was similar to previously reported values for chicken skeletal muscles (Misra *et al*., 1980; Al-Najdawi and Abdullah, 2002). Independent of the housing system, muscle [K^+] was 5.2 mEq/L and 4.7 mEq/L higher in LL compared with SH ($P < 0.02$) and LB ($P < 0.03$) hens, respectively. Within CC housing, muscle [K^+] was 6.2 mEq/L higher in LL compared with SH hens ($P < 0.001$). In the FC system, muscle [K^+] trended 5.8 mEq/L higher in LL, compared with LB hens ($P = 0.099$). Housing treatment differences were observed in which the muscle [K^+] was 3.5 mEq/L higher in FC (117.4 ± 1.28) compared with CC (113.9 ± 0.9) ($P < 0.04$).

3.3.3.2 *Muscle concentrations of Cl^- and lactate$^-$*

Muscle [Cl^-] (Figure 3-3A) varied between 7.9 – 9.2 mEq/L and was similar to previous values reported in rodent and chicken muscles (Misra *et al.*, 1980; Lindinger *et al.*, 1987). Muscle [Cl^-] did not differ between hen strains ($P > 0.18$) or between the CC and FC housing systems ($P > 0.85$).

Muscle [lactate$^-$] (Figure 3-3B) varied between 51.0 – 65.7 mEq/L and was similar to previously reported values in poultry muscles (Berri *et al*., 2007; Debut *et al*., 2005; Le Bihan-Duval *et al.*, 2008). Muscle [lactate$^-$] was 11.8 and 12.5 mEq/L higher in LB hens compared with SH ($P < 0.005$) and LL ($P < 0.005$) hens, respectively, independent of the housing system. In the CC system, muscle [lactate$^-$] was 14.6 and 13.4 mEq/L higher in LB hens compared with SH ($P < 0.005$) and LL ($P < 0.005$) hens, respectively. In the FC system, muscle [lactate$^-$] trended 11.7 mEq/L higer in LB compared with LL hens ($P = 0.06$). Muscle [lactate$^-$] did not differ between CC (56.3 ± 1.9) and FC (58.1 ± 2.1) housing systems ($P > 0.53$).

3.3.3.3 *Sums of strong base cations and strong acid anions*

Strain differences were noted for $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$ (Figure 3-4A). Independent of the housing system, $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$ was 10 mEq/L greater in LL hens compared with SH hens ($P < 0.02$). In the CC system, LL trended 8.2 mEq/L ($P = 0.06$) higher and LB trended 7.3 mEq/L ($P = 0.09$) higher compared with SH hens. In the FC housing system, LL trended 11.6 mEq/L higher than SH hens ($P = 0.07$). Housing treatment differences were observed in which the $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$ was 10 mEq/L greater in the FC compared with the CC housing system ($P < 0.005$).

Strain differences were also noted for $\sum[Cl^{-}]+[lactate^{-}]$ (Figure 3-4B). When housing treatments were combined, $\sum[Cl^{-}]+[lactate^{-}]$ was 11.1 and 12.6 mEq/L greater in LB hens compared with SH ($P < 0.01$), and LL ($P < 0.005$) hens, respectively. Within the CC system, the $\sum[Cl^{-}]+[lactate^{-}]$ was 13.1 and 14.1 mEq/L greater in LB hens compared with LL ($P < 0.01$) and SH ($P < 0.01$) hens, respectively. Within the FC system, the $\sum[Cl^{-}]+[lactate^{-}]$ trended 12.1 mEq/L higher ($P = 0.06$) in LB hens compared with LL hens. The $\sum[Cl^{-}]+[lactate^{-}]$ did not differ between CC (64.8 ± 1.9) and FC (66.5 ± 2.2) housing systems ($P > 0.56$).

3.3.4 [SID], $[A^{-}]$ and $[HCO_3^{-}]$

The [SID] varied between 148.9 – 175.7 mEq/L (Figure 3-5A). The [SID] trended 11.5 mEq/L higher in LL, compared with SH hens ($P = 0.08$); it was 16.6 mEq/L higher in LL compared with LB hens ($P < 0.03$). In the CC system, the [SID] trended 14.2 mEq/L higher in LL hens, compared with LB hens ($P = 0.07$). In the FC system, the [SID] trended 19.0 mEq/L higher in LL hens, compared with LB hens ($P = 0.10$). Muscle [SID] was not significantly different between CC (156.0 ± 3.2) and FC (164.1 ± 4.2) housing systems ($P = 0.13$).

The $[A^-]$ (Figure 3-5B) varied between 140.6 – 163.5 mEq/L and was not significantly different between strains ($P > 0.28$), or between CC (144.3 ± 5.9) and FC (148.9 ± 5.8) housing systems ($P > 0.58$).

Muscle bicarbonate ($[HCO_3^-]$) varied between 12.2 – 20.9 mEq/L (Figure 3-5C) and trended 4.7 mEq/L higher in LL compared with LB hens ($P = 0.06$). Housing treatment differences were observed, with muscle $[HCO_3^-]$ being 4.8 mEq/L greater in the FC compared (18.7 ± 1.3) to the CC (13.9 ± 1.3) housing system ($P < 0.03$).

3.3.5 [Atot] and PCO_2

Total muscle protein ($P > 0.55$), $[A_{tot}]_{protein}$ ($P > 0.55$) and $[A_{tot}]_{weak\ acids}$ ($P > 0.82$) (Table 3-2) did not differ between strains or between the CC and FC housing systems ($P > 0.64$). Muscle $[A_{tot}]$ (Figure 3-6A) varied between 250.8 – 279.4 mEq/L in hen *pectoralis major* and did not differ between strains ($P > 0.68$), or between CC (262.2 ± 14.4) and FC (269.0 ± 14.3) housing systems ($P > 0.75$). $[A_{tot}]_{protein}$ and $[A_{tot}]_{weak\_acids}$ accounted for 20 ± 1.2% and 80 ± 1.2% of $[A_{tot}]$, respectively.

Muscle PCO_2 varied between 88.7 – 129.1 mmHg (Figure 3-6B) and did not differ between hen strains ($P > 0.79$). However, housing differences were noted for muscle PCO_2. When all strains were combined, muscle PCO_2 was 33.7 mmHg higher in the FC (127.2 ± 8.6) system compared to the CC (93.5 ± 8.7) system ($P < 0.03$).

3.3.6 Muscle pH and $[H^+]$

3.3.6.1 *Measured muscle pH and $[H^+]$*

Measured pH ($pH_{measured}$) varied between 6.547 – 6.679 (Figure 3-7A), while the corresponding $[H^+]$ ($[H^+]_{measured}$) varied between 209 – 284 nEq/L (Figure 3-7B). There was a

strain effect for muscle $pH_{measured}$ and $[H^+]_{measured}$. When housing treatments were combined, muscle $pH_{measured}$ was 0.099 lower for LB compared with LL hens ($P < 0.05$) (Figure 3-7A) and the corresponding $[H^+]_{measured}$ was 54 nEq/L higher in LB compared with LL hens ($P < 0.05$) (Figure 3-7B). Within the FC system, $pH_{measured}$ trended 0.132 lower (Figure 3-7A) and $[H^+]_{measured}$ trended 74 nEq/L higher (Figure 3-7B) ($P = 0.08$) in LB compared with LL hens. Neither $pH_{measured}$ nor $[H^+]_{measured}$ differed between the CC and FC systems ($P > 0.92$).

3.3.6.2 *Calculated muscle pH and $[H^+]$*

Muscle $pH_{calculated}$ and $[H^+]_{calculated}$ varied between 6.569 – 6.708 (Figure 3-8A) and 196 – 270 nEq/L (Figure 3-8B), respectively. There were strain effects for muscle $pH_{calculated}$ and $[H^+]_{calculated}$. When housing treatments were combined, muscle $pH_{calculated}$ was 0.105 lower ($P < 0.05$) (Figure 3-8A) and $[H^+]_{calculated}$ was 54 nEq/L higher for LB as compared with LL hens ($P < 0.05$) (Figure 3-8B). In the FC system, $pH_{calculated}$ trended 0.139 lower (Figure 3-8A) and $[H^+]_{calculated}$ trended 74 nEq/L higher (Figure 3-8B) ($P = 0.08$) for LB as compared with LL hens. Neither $pH_{calculated}$ nor $[H^+]_{calculated}$ differed between the CC and FC systems ($P > 0.92$).

3.3.7 Regression analysis

Muscle $[H^+]_{calculated}$ was linearly related to $[H^+]_{measured}$ ($P < 0.001$) (Figure 3-9A). Muscle $[H^+]_{calculated}$ accounted for 99.7% of the variation in $[H^+]_{measured}$. Muscle $pH_{calculated}$ was also linearly related to $pH_{measured}$ and accounted for 99.6% of the variation in $pH_{measured}$ ($P < 0.001$) (Figure 3-9B).

3.4 Discussion

The present study investigated post-mortem poultry *pectoralis major* $[H^+]$ within the conceptual and empirical framework of the physicochemical model derived by Stewart (Stewart,

1981; Stewart, 1983). The Stewart model proved to be exceptional, accounting for > 99% of the variation in *pectoralis major* [H^+] and its corresponding log transformation, pH (Figure 3-9). The novel findings of this study are two-fold. First, variations in the [SID] systems-independent variable accounted for most or all of the observed differences in [H^+] between bird strains. Second, although [H^+] did not differ between CC and FC systems, the relative contributions of the three systems-independent variables differed between housing systems.

3.4.1 Application of the Stewart model to evaluate differences in meat pH

Although glycolytic potential has been correlated with lactate accumulation (El Rammouz *et al.*, 2004; Zhang *et al*., 2009), and lactate accumulation undoubtedly accounts for a significant portion of post-mortem acidification of meat (Monin & Sellier 1985; El Rammouz *et al.*, 2004; Berri *et al*., 2007; Le Bihan-Duval *et al.,* 2008; England *et al.*, 2014; England *et al.*, 2016) significant dissociations have been reported indicating the involvement of other factors. Examination of a report by Monin and Sellier (1985) reveals that although lactate was ~11 mEq/L lower in a variety of muscles in Hampshire compared to Pietrain pigs, the corresponding ultimate pH (pH_u) was lower. Similar dissociations were reported in breast and thigh muscles of broilers exposed to prolonged stress (Zhang *et al.*, 2009). Zhang *et al*. (2009) showed, for example, that transport stress caused lactate to increase by 9 mEq/L without a corresponding change in pH_u. In a more recent study by Matarneh *et al.* (2015), initial pH (pH_i) was the same within *longissimus lumborum* of wild-type and AMPKγ3^{R200Q} pigs even though lactate concentration was 10 mEq/L higher in wild-type pigs. Lower pH_u reported for AMPKγ3^{R200Q} pigs amounted to a net increase in [H^+] on the order of 1,300 nEq/L, but it occurred in the absence of differences in lactate.

A comparison of hen strains examined in the present study also revealed dissociations between glycolytic potential and lactate accumulation, and between lactate and $[H^+]$ accumulation that can be reconciled within the context of the Stewart model. Whereas greater glycolytic potential of LB, compared with LL, (Figure 3-1C) corresponded to the accumulation of lactate (Figures 3-1B and 3-3B) and $[H^+]$ (Figure 3-7B), the same comparison between SH and LL revealed a moderate dissociation between these variables. Greater glycolytic potential within SH, compared with LL, did not induce a significant increase in lactate content, yet $[H^+]$ still demonstrated an absolute increase of 29 nEq/L (Figure 3-7B). Using the Stewart equation (eq. 8) it is possible to calculate that, in this instance, 100% of the 29 nEq/L rise in $[H^+]$ in SH was attributed to a reduction in the [SID] that resulted solely from a 10 mEq/L reduction in the $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+]$ (Figure 3-4A).

In contrast, 85% of the 54 nEq/L increase in $[H^+]$ within LB, compared with LL hens (Figure 3-7B), was attributed to an 16.6 mEq/L difference in the SID (eq. 8) (Figure 3-5A). In this instance 75% of the decline in the [SID] in LB hens was attributed to the greater lactate accumulation (Figure 3-3B and 3-4B); the remaining 25% of the decline in the [SID] was attributed to slight decrease in the $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+]$ (Figure 3-4A). The remaining 15% of the 54 nEq/L increase in $[H^+]$ within LB, compared with LL hens, was attributed to a 5.7 mEq/L increase in A_{tot} in LB hens

Another major finding of the present study was that hens housed in FC systems achieved acid-base balance in a manner that differed from hens housed in the CC system. The fact that P_{CO_2} was 33.7 mmHg higher within hens housed in the FC system (Figure 3-6B) but did not demonstrate correspondingly higher $[H^+]$ could be explained by compensatory adaptive changes in one or both of the other two systems-independent variables. Using eq. 8 and the mean values

of the three systems-independent variables for the CC and FC housing systems, it is possible to calculate that the potential for P_{CO_2}-induced elevation of the $[H^+]$ in hens housed in the FC system was counteracted by an 8 mEq/L rise in the [SID], the latter having resulted from greater intramuscular sequestration of strong base cations (Figure 3-4A) but independent of variations in lactate accumulation (Figures 1B and 3B) or glycolytic potential (Figure 3-1C).

3.4.2 [SID]

In the present study, variations in the [SID] between hen strains accounted for most or all of the observed differences in $[H^+]$. This indicates that post-mortem muscle $[H^+]$ is significantly affected by heritable differences in ion transport capacity expressed during the ante-mortem period. The fact that strain differences in the $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+]$ were attributed to variations in muscle $[Na^+]$ and $[K^+]$ suggests differences in the content or specific activities of the Na^+/Ca^{2+} exchanger (NCX) and Na^+/K^+-ATPase pump (Na^+/K^+-pump). In this regard, it is interesting to consider the potential impact of stress-induced elevation of glucocorticoids, especially in light of the fact that White Leghorns demonstrate a greater stress-induced increase in glucocorticoids compared with Brown layer strains (Fraisse & Cockrem 2006). Smith and Smith (1994) showed that expression of NCX was down-regulated by glucocorticoids, while Ravn and Dorup (1997) reported muscle K^+ loss was associated with prolonged elevation of glucocorticoid levels. Thus, lower NCX expression in SH hens probably accounted for both lower $[Na^+]$ and lower $[K^+]$ in those birds. This hypothesis is supported by reports that the Na^+/K^+-pump is dependent on $[Na^+]$ and ATP for activity (Therien & Blostein, 2000) and the K_m for Na^+ is estimated to be 12-33 mM (Zahler *et al*., 1997), where it is optimally positioned to regulate the Na^+/K^+-pump activity in direct proportion to $[Na^+]$ in the current study.

Ante-mortem accumulation of muscle lactate is the net result of an imbalance between production (*i.e.*, glycogenolysis) and removal (Putman *et al.,* 1995), by either monocarboxylic lactate transporter (MCT)-dependent extrusion into the venous blood (Bonen *et al*., 1998) or metabolism within muscle fibres (Putman *et al*., 1998, 2003). In contrast, in the absence of blood flow and oxygen, post-mortem lactate accumulation is only attributed to continued glycogenolysis (Pösö & Puolanne, 2005) and must be proportional to the initial glycogen content (Newsholme & Start, 1973). The standardized handling procedures used in the present study should have minimised ante-mortem glycogenolysis, and thus it seems reasonable to conclude that greater lactate accumulation in LB resulted, at least in part, from greater post-mortem glycogen breakdown. However, it is also possible that the greater lactate accumulation in LB hens resulted from lower rates of ante-mortem lactate extrusion secondary to lower sarcolemmal MCT content. Indeed, expression of MCT has been shown to differ between breeds of various species (Mykkänen *et al.,* 2010; Parkunan *et al*., 2015) and MCT expression is reportedly elevated in response to stress (Parkunan *et al*., 2015). It is also possible that greater stress responses by SH and LL hens accelerated ante-mortem lactate oxidation (Gleeson *et al*., 1993).

Variations in the $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$ that were observed between housing systems indicates that post-mortem muscle $[H^{+}]$ has the potential to be affected by ante-mortem conditions including the opportunity for movement within production facilities. Greater sequestration of strong base cations within hens housed in the FC system indicates that those hens possessed a greater capacity to defend against post-mortem acidification. We suggest that the greater $\sum[Ca^{2+}]+[Mg^{2+}]+[Na^{+}]+[K^{+}]$ observed in the FC housing system probably resulted from increased activity of hens who expressed natural behaviours on a daily basis. This is supported by previous reports showing that activity increased the expression of Ca^{2+}-binding

proteins (Ohlendieck *et al*., 1999), Ca^{2+} storage volume (Murphy *et al*., 2009; Fryer & Stephenson, 1996), Mg^{2+}-ATP content (Green *et al*., 2000), K^+ uptake (Green *et al*., 2000), NCX expression (Bueno *et al*., 2010) and capillary volume (Škorjanc *et al.*, 1998).

3.4.3 $[A_{tot}]$

Muscle $[A_{tot}]$ represents the total concentration of undissociated plus dissociated nonvolatile organic weak acids that behave as classical weak acid buffers (Stewart, 1981; Stewart 1983). A major component of $[A_{tot}]$ is formed by proteins that possess a net negative charge of 2.45 g/dL within the pH range observed in the current study (Jones, 1987). Within complex structural and metabolic proteins, the ionizing amino acid side chain of histidine (pKa = 6.04) is ideally positioned to buffer H^+ within the pH range typically observed in ante- and post-mortem muscle; additional contributors may also include free amino acids with isolelectric points (pI) within the same range. The second major component of $[A_{tot}]$ includes an as yet undetermined number of phosphorylated metabolites (pKa ~ 6.1), as well as ATP, ADP (pKa ~ 6.5) and AMP (pKa ~ 6.1) (Jones, 1987; Lindinger & Heigenhauser; 1991; Lindinger, 1995). In the present study we measured the first component, total protein, but did not measure the phosphorylated metabolites because the latter would have encompassed an unreasonably large number of weak acids and associated K_A values. It also proved to be unnecessary because the Stewart model allowed for the derivation of single values for K_A and $[A_{tot}]$.

In order to determine $[A_{tot}]$, we first calculated $[A^-]$ using the strong ion electroneutrality equation (eq. 5), and experimentally determined values of [SID] and $[HCO_3^-]$. This allowed us to then calculate $[A_{tot}]$ (eq. 6) based on measured $[H^+]$ and the dissociation constant for weak acids, K_A, which is equal to 3.0×10^{-7} Eq/L (*i.e.*, $pK_A = 6.6$) (Stewart, 1981; Stewart, 1983). This approach is supported by a fundamental principle of the Stewart model which states that within

biological systems the value of K_A remains constant even when the [SID] and $[A_{tot}]$ systems-independent variables change (Stewart, 1981; Stewart, 1983). It is also supported by the findings of Stämpfli *et al.* (2006) who used CO_2 tonometry to estimate that the K_A of avian plasma was between 2.15 x 10^{-7} and 3.25 x 10^{-7} Eq/L (*i.e.*, $pK_A \simeq 6.5 - 6.7$). This approach proved to be reliable when the resulting $[A_{tot}]$ values were used to calculate $[H^+]$ (Figure 3-9A).

An important finding of the present study was that muscle protein concentration represented only 20% of $[A_{tot}]$ and was remarkably constant across hen strains and between housing conditions. In our study, the remaining 80% of $[A_{tot}]$ was attributed to organic weak acids and was also constant across strains and between housing conditions. Because the $[A_{tot}]$ systems independent variable is considerably more constant than [SID] and P_{CO_2}, its quantitative significance is often overlooked. Using the Stewart equation and our empirically determined values of $[A_{tot}]$, [SID] and P_{CO_2} for the three hen strains, it is possible to calculate that reducing $[A_{tot}]$ by half would result in a 75% decrease in $[H^+]$ thus highlighting the importance of applying rigorous quantitative modelling to determine the contributions of $[A_{tot}]$. Such considerations may prove important when, for example, attempting to establish the cause of Pale Soft Exudative (PSE) meat. It is plausible that it is not the increase in $[H^+]$ that induces protein denaturation and lower water hold capacity (WHC) within PSE meat (Ahn & Maurer, 1990; Barbut *et al.*, 2005; Berri *et al.*, 2005; Le Bihan-Duval *et al.*, 2008), but rather that excessive protein breakdown and water loss cause $[A_{tot}]_{protein}$ and $[A_{tot}]_{weak\ acids}$ to rise which in turn is the actual cause of increased $[H^+]$ in PSE meat.

3.4.4 P_{CO_2}

Greater muscle P_{CO_2} observed within the *pectoralis major* of hens housed in the FC system of the present study is consistent with ante-mortem adaptive changes having occurred

that enhanced the capacity for oxidative CO_2 production and elevated myocellular oxygen storage capacity. Branciari *et al.* (2008) showed that the greater activity of Leghorns allowed daily access to grass paddock was sufficient to induce the transition of fast-glycolytic to slower more oxidative fibers within the *pectoralis major*, while Castellini *et al.* (2002) reported the same production conditions induced a 20% increase in haeme iron. Thus, most of the post-mortem CO_2 accumulation that we observed in hens housed in the FC system probably resulted from new ante-mortem steady-state CO_2 production. It is also plausible that greater Mb-O_2 continued to stimulate Kreb's cycle flux, further contributing to CO_2 accumulation early in the post-mortem period. In the present study, the corresponding increase of muscle [HCO_3^-] must be considered obligatory given the rapidly reversible nature of carbonic anhydrase (Geers & Gros, 2000).

3.5 Conclusion

The results of this study demonstrate the utility of comprehensive physicochemical modelling to investigate the factors responsible for variations in poultry meat [H^+] within an agricultural production setting. The Stewart model allowed calculation of [H^+] with a very high degree of accuracy when compared with measured values of [H^+]. It also provided the conceptual framework to identify factors which, acting through the [SID], were responsible for differences in [H^+] between hen strains that included sequestration of strong base cations and excess lactate accumulation. Application of the model to evaluate the experimental effect of CC versus FC housing systems revealed that although *pectoralis major* muscles of hens housed in the FC system contained higher levels of P_{CO_2}, post-mortem acidification was countered by greater sequestration of strong base cations in those hens. It is suggested that the housing treatment differences resulted from activity-induced adaptations within hens housed in the FC system.

Table 3-1

Water content in *pectoralis major* muscles of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error.

Variable	Housing Treatment	Strains Combined	SH	LL	LB
wet-to-dry weight ratio	Treatments Combined	—	3.81 ± 0.03	3.82 ± 0.03	3.82 ± 0.03
	CC	3.82 ± 0.03	3.78 ± 0.05	3.84 ± 0.04	3.82 ± 0.04
	FC	3.82 ± 0.03	3.84 ± 0.04	3.80 ± 0.05	3.82 ± 0.05
	P - Housing	0.83	0.39	0.53	0.95
total tissue water (ml/g ww)	Treatments Combined	—	0.738 ± 0.002	0.738 ± 0.002	0.738 ± 0.002
	CC	0.738 ± 0.002	0.735 ± 0.003	0.740 ± 0.003	0.738 ± 0.003
	FC	0.738 ± 0.002	0.740 ± 0.003	0.737 ± 0.003	0.738 ± 0.003
	P - housing	0.81	0.38	0.54	0.95

Table 3-2

Total protein, $[Atot]_{protein}$ and $[Atot]_{weak\ acids}$ in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error.

Variable	Housing Treatment	Strains Combined	SH	LL	LB
total protein (g/dL TTW) n = 42	Treatments Combined	—	21.37 ± 1.69	18.79 ± 1.80	19.72 ± 1.76
	CC	20.38 ± 1.52	22.81 ± 2.54	20.34 ± 2.53	18.00 ± 2.36
	FC	19.54 ± 1.37	19.92 ± 2.64	17.25 ± 2.65	21.45 ± 2.56
	P - housing	0.68	0.47	0.41	0.32
$[Atot]_{protein}$ (mEq/L) n = 42	Treatments Combined	—	52.35 ± 4.14	46.04 ± 4.40	48.33 ± 4.32
	CC	49.94 ± 3.71	55.89 ± 6.21	49.82 ± 6.21	44.09 ± 5.79
	FC	47.87 ± 3.36	48.81 ± 6.47	42.25 ± 6.49	52.56 ± 6.27
	P - housing	0.68	0.47	0.42	0.32
$[Atot]_{weak\ acids}$ (mEq/L) n = 42	Treatments Combined	—	208.54 ± 16.02	217.71 ± 17.68	222.36 ± 16.57
	CC	211.47 ± 14.03	212.89 ± 23.48	198.69 ± 23.25	222.84 ± 20.51
	FC	220.93 ± 14.01	204.19 ± 25.37	236.73 ± 27.57	221.87 ± 24.96
	P - housing	0.65	0.82	0.32	0.98

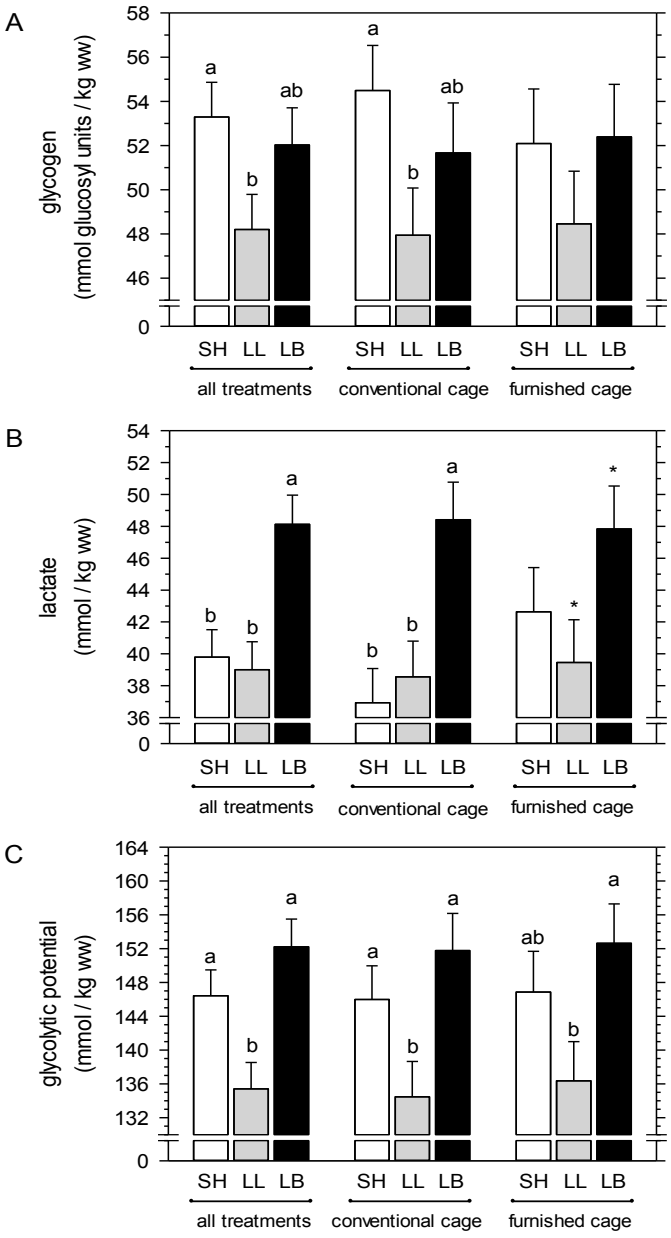

Figure 3-1. Contents of (A) glycogen and (B) lactate, and (C) glycolytic potential in *pectoralis major* muscles of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P < 0.1$).

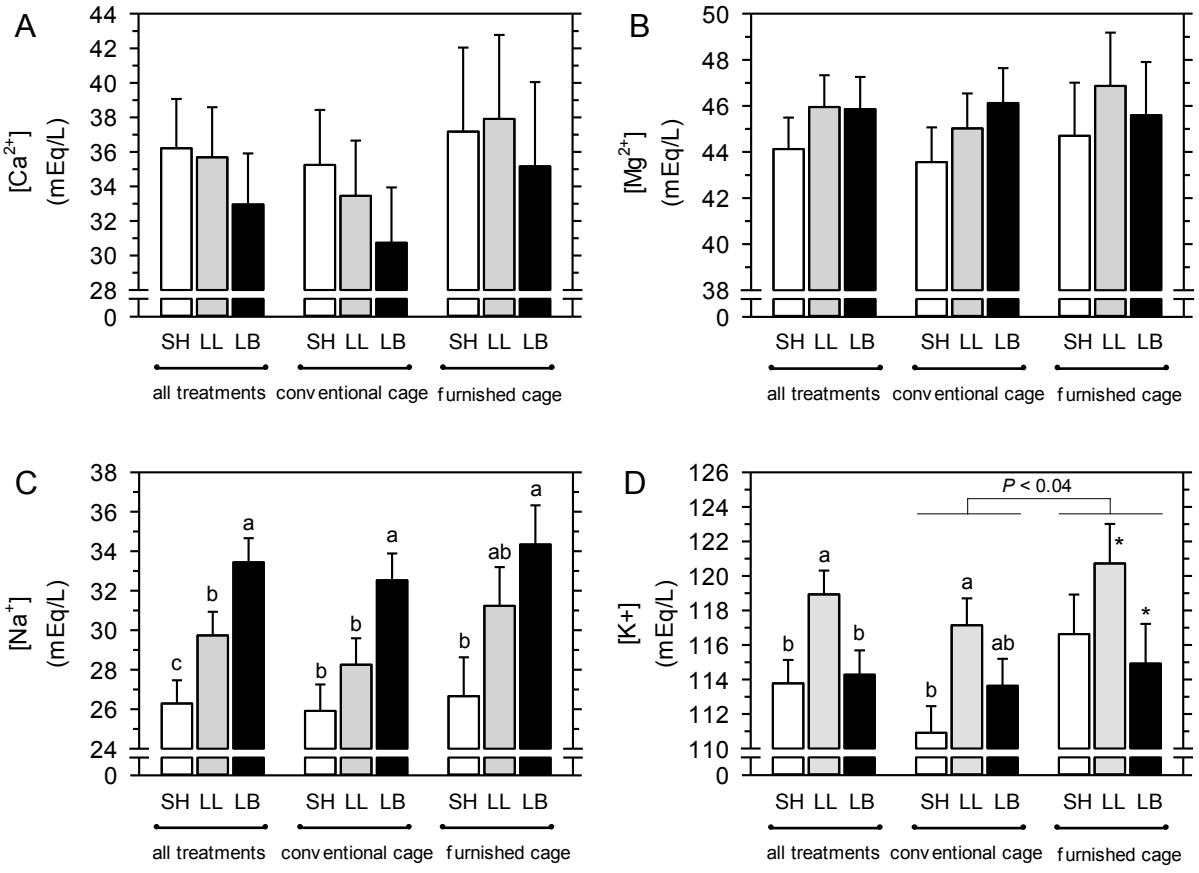

Figure 3-2. Concentrations of (A) calcium, (B) magnesium, (C) sodium and (D) potassium in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P < 0.1$).

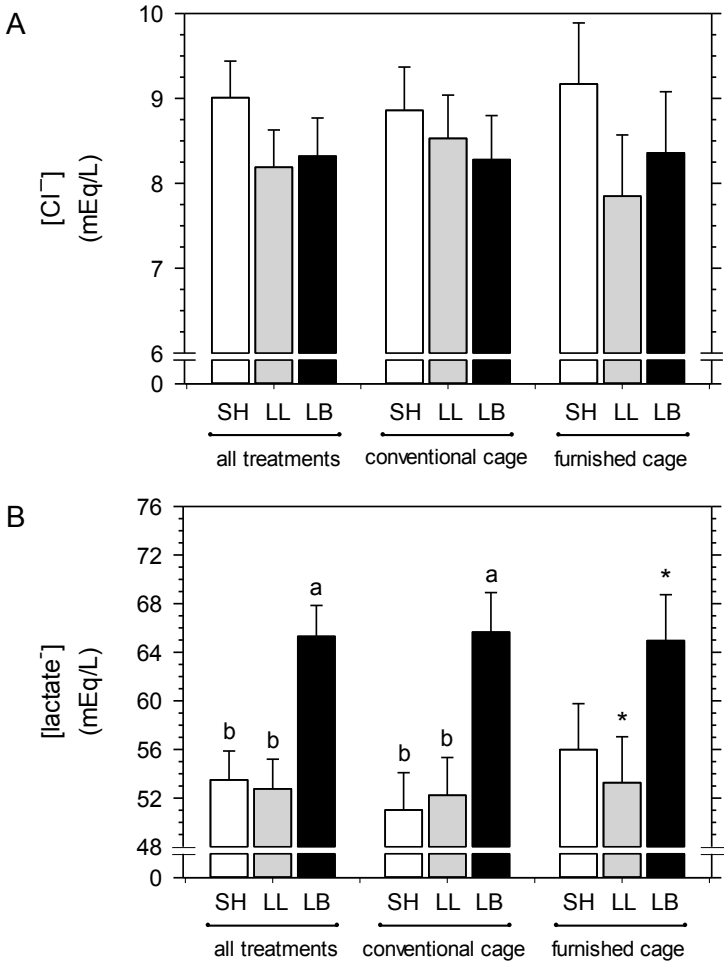

Figure 3-3. Concentrations of (A) chloride and (B) lactate in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means within a housing condition ($0.05 < P < 0.1$).

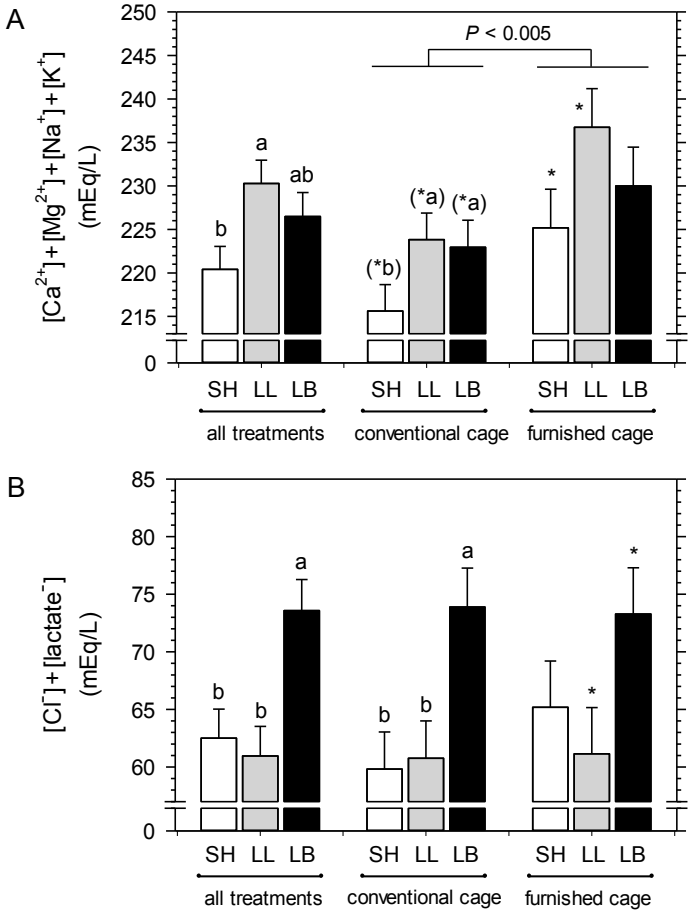

Figure 3-4. The sum of (A) strong base cations and (B) sum of strong acid anions in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P < 0.1$).

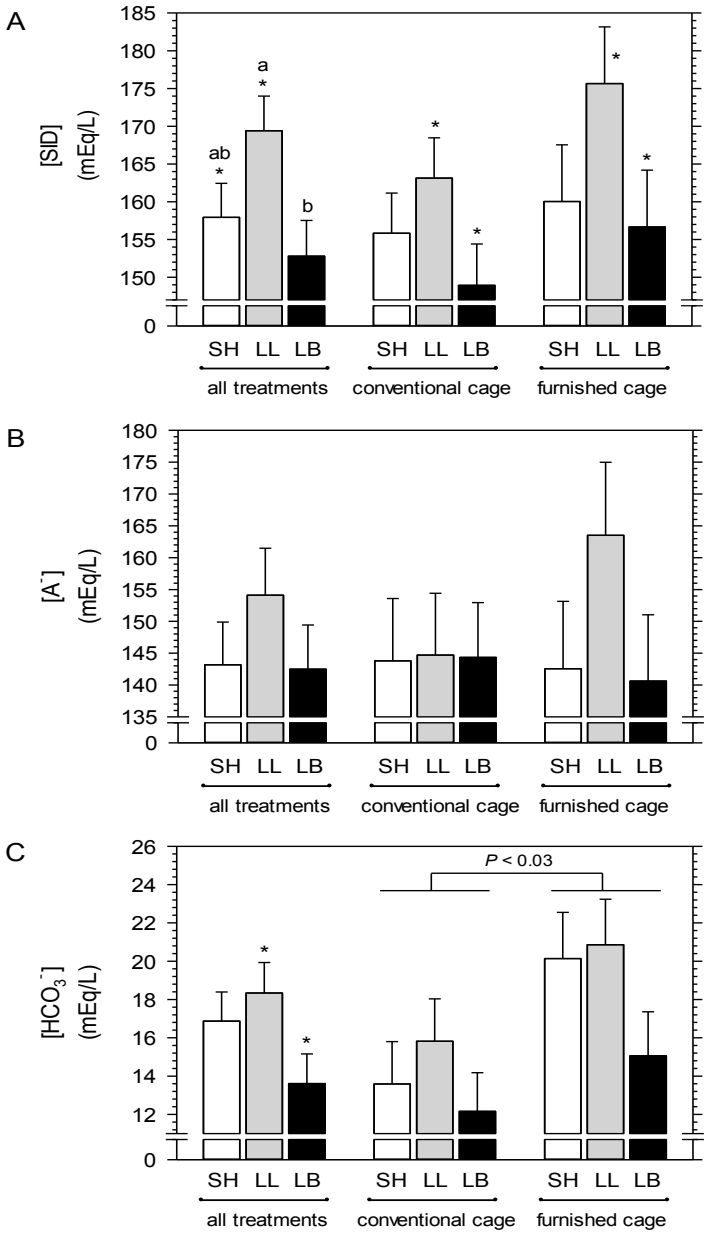

Figure 3-5. The (A) SID, (B) anion gap [A^-], and (C) HCO_3 in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P < 0.1$).

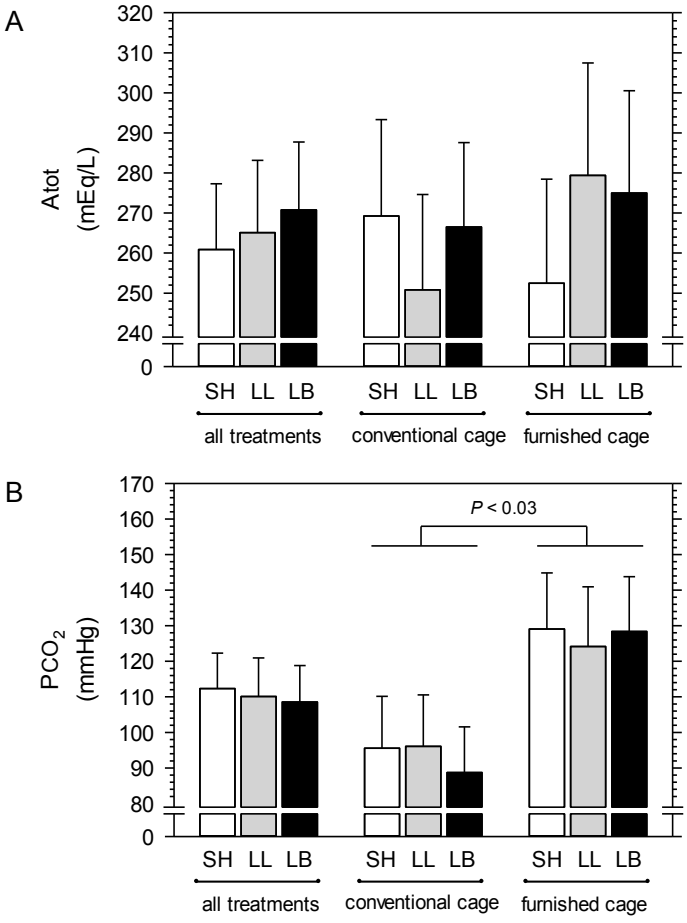

Figure 3-6. The (A) [A_{tot}] and (B) PCO_2 in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. Significantly differences are indicated by *P*-values on the graph.

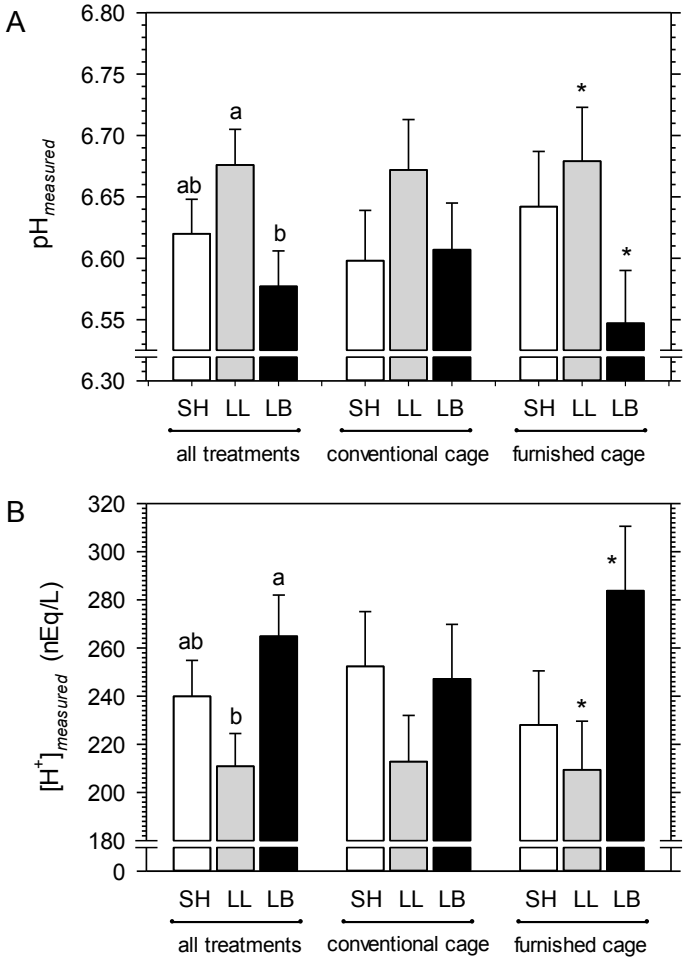

Figure 3-7. Measured (A) pH and (B) $[H^+]$ in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P \leq 0.1$).

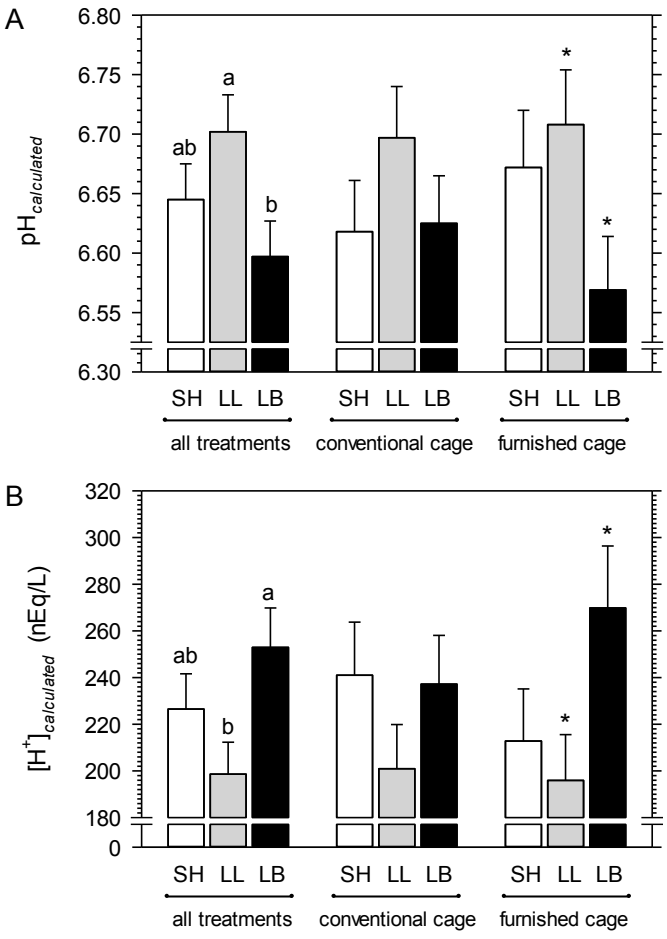

Figure 3-8. Calculated (A) pH and (B) $[H^+]$ in *pectoralis major* of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages). Data are least square means ± standard error. [a,b] Least square means with different letters are significantly different ($P \leq 0.05$) between hen strains or within a housing treatment. * Denotes a trend between two least square means ($0.05 < P \leq 0.1$).

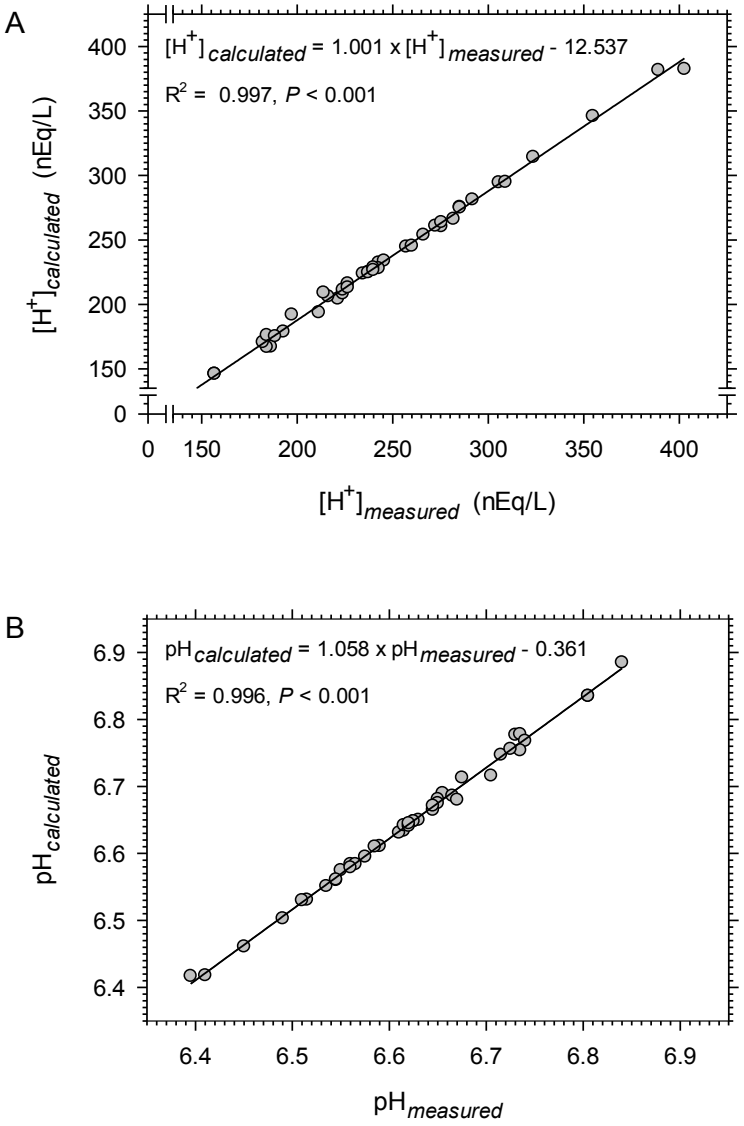

Figure 3-9. Regression analysis of (A) measured and calculated [H^+] and (B) measured and calculated pH in pectoralis major of SH (Shaver White), LL (Lohmann Lite), and LB (Lohmann Brown) housed in CC (conventional cages) or FC (furnished cages) (n=42).

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CHAPTER 4: FINAL DISCUSSION

4.1 Summary of Results

Examining meat quality and the physicochemical properties of post-mortem muscle provides essential information regarding the effects of ante-mortem conditions on muscle physiology and biochemistry. Traditional meat quality studies contain important information on the visual and sensory characteristics of muscle. However, although meat quality studies typically include muscle pH determination, they do not explain the underlying factors that lead to the muscle pH values. Use of the Stewart Model (Stewart 1981; Stewart, 1983) to calculate muscle $[H^+]$ can accurately account for the fundamental cellular factors that determine muscle pH, and ultimately meat quality, while also providing important information on muscle biochemistry and overall function.

The purpose of the research presented in Chapter 2 was to evaluate the effects of both laying hen housing environment and genetic strain on meat quality. It was hypothesized that hens housed in FC who have greater opportunity for movement and the ability to express highly motivated behaviour would exhibit improved meat quality compared to conventionally-caged hens, and that differences in stress responses between genetic strains would lead to differences in meat quality. To assess meat quality in this study, *Pectoralis major* muscles from two flocks of three strains of laying hens (Shaver White (SH), Lohmann Lite (LL) and Lohmann Brown (LB)) housed in either conventional cages (CC) or furnished cages (FC) were analyzed for muscle pH, colour and shear force (SF).

The overall lack of housing differences in muscle pH, colour and SF reported in Chapter 2 suggested that hens in both housing systems likely experienced physiological stress that similarly affected meat quality. In the CC housing system, hens experience stress as a result of

physical and behavioural restriction (Appleby *et al*., 2002) and, while hens housed in the FC system are permitted increased space and the amenities necessary to express highly motivated natural behaviours, they must contend with larger group housing which results in social stress (Keeling *et al*., 2003).

Differences in meat quality between genetic strains reported in Chapter 2 suggested increased stress was experienced by SH and LL hens as compared with LB hens. The lower initial muscle pH (pH_{17}) and ultimate muscle pH (pH_u) in LB hens, as compared with LL and SH hens, and greater pH decline (ΔpH) in LB hens as compared with SH hens suggested that peri-mortem glycogen content may have been lower in SH and LL hens as compared with LB hens. Redder muscle for LL birds than for SH and LB hens, and higher muscle SF for SH hens as compared with LL and LB birds could also be the result of lower peri-mortem muscle glycogen content and greater ante-mortem glycogenolysis for SH and LL as compared to LB hens. To further investigate differences in muscle pH and to gain a better understanding of the factors contributing to the post-mortem muscle pH decline, a second study was undertaken (Chapter 3).

The purpose of the study reported in Chapter 3 was to investigate *Pectoralis major* muscle glycogen content and the three systems-independent variables that affect muscle $[H^+]$ according to the Stewart model (Stewart, 1981; Stewart, 1983). The accuracy of the Stewart model was also evaluated by comparing measured muscle $[H^+]$ values to values of muscle $[H^+]$ as calculated using the Stewart equation (eq. 8, Chapter 3). It was hypothesized that the three systems-independent variables would differ between genetic strains and between housing environments in a manner that would account for variations in measured muscle $[H^+]$. The hypothesis presented in Chapter 2, that variations in muscle glycogen content were the cause of the strain differences in muscle pH, colour and SF was largely refuted in Chapter 3, as

dissociations emerged between lactate (Lac^-) accumulation and [H^+]. The three systems-independent variables accounted for variations in muscle [H^+] and its corresponding log transformation, pH.

In order to examine the three systems-independent variables and to calculate muscle [H^+] using the Stewart model (Chapter 3), muscle samples were analyzed for the strong ions Ca^{2+}, Mg^{2+}, Na^+, K^+, Cl^- and lactate (Lac^-), which were then used to calculate the strong ion difference ([SID]). Total weak acids ([A_{tot}]) and P_{CO_2} were also determined. Regression analyses (Chapter 3) revealed that values of muscle [H^+] that were calculated using the Stewart equation (eq. 8, Chapter 3) accounted for > 99% of the variation in measured muscle [H^+] and its corresponding log transformation, pH. Importantly, variations in the systems-independent variable [SID] accounted for most or all of the differences in muscle [H^+] between genetic strains: (1) Greater muscle [H^+] in LB hens, as compared with LL hens, was attributed to lower muscle [SID] that resulted from increased Lac^- accumulation in LB hens. (2) In contrast, an absolute increase in [H^+] within SH hens, as compared with LL, hens was attributed to lower [SID] that resulted from lower $\sum([Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+])$ in SH hens.

While there were no differences in muscle [H^+] between housing conditions, the results showed that in FC the potential for greater post-mortem acidification by higher P_{CO_2} was defended by a rise in [SID] that resulted from increased sequestration of strong base cations [*i.e.*, greater $\sum([Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+])$]. It is suggested that the increase in muscle P_{CO_2} and $\sum([Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+])$ within FC hens resulted from the greater opportunity for movement. Although hens housed in both CC and FC likely experienced different environmental stressors that affect meat similarly, the increased activity of hens housed in FC may have improved overall muscle function and health. Collectively, the research outcomes reported in Chapter 3

demonstrate that the Stewart Model was remarkably accurate in predicting post-mortem [H^+] within laying hen *Pectoralis major* muscles. Moreover the Model provided the essential conceptual and empirical framework that allowed causal factors that determine [H^+] to be investigated.

4.2 Significance of this Research

The research presented in this thesis demonstrates how laying hen housing environment and genetic composition can affect measures of meat quality and physicochemical properties of muscle. In Chapter 2, meat quality characteristics were measured and compared for three laying hen strains housed in either CC or FC. This study provided novel information on how housing environment affected muscle properties, as shown through variations in muscle pH, colour, and shear force. Additionally, this study provided important information on the suitability of each of the three strains to the different housing environments. Meat quality has not previously been investigated for laying hens housed in these particular cage systems and this research is essential to the current and on-going discussions on the state of laying hen housing around the world.

Past research has showed that hens housed in CC experience increased stress as evidenced through the expression of stereotypic behaviour and aggression (Appleby *et al*., 2002; Yue and Duncan, 2003). The increased stress for hens housed in CC is the result of spatial and behavioural restriction (Appleby *et al*., 2002) and these welfare issues for hens housed in CC are recognized worldwide. As a result, these cages have been banned in certain countries and are currently being phased out in many others (Prevention of Farm Animal Cruelty Act, 2008; CEC, 1999; SFS, 1998; SAWO, 1981). Furnished cage systems are expected to be a suitable alternative to CC and are expected to be beneficial to hen well-being as they provide increased space and amenities that allow hens to engage in highly motivated nesting, dust-bathing,

foraging and perching behaviours. Unfortunately, FC typically house larger group sizes than CC which causes social stress. Consequently, the research presented in this thesis generally lacked large differences in meat quality between CC and FC. The presence of stress in both systems and consequent lack of housing differences in meat quality were contrary to the expectation that FC hens would exhibit improved meat quality characteristics over hens housed in CC.

Despite the presence of stress in both systems, housing differences were demonstrated in Chapter 3 that suggested FC housing systems may benefit hens physically. Increased opportunity for locomotory behaviour was expected to be the cause of greater cation sequestration and increased CO_2 production for hens housed in FC as compared with hens housed in CC. These differences suggested enhanced metabolic capabilities for FC hens and while these effects were likely counteracted by the presence of stress in both housing systems, they could still have important systemic implications for overall muscle health. This is especially true as humeral bone strength of FC hens is greater than those of hens housed in CC (Jendral, 2008; Jendral, 2012) and it would therefore be expected that muscle health would be positively affected as well. One of the main reasons laying hen meat is not produced for human consumption is that weak hen bones typically splinter throughout the meat at processing (Gregory and Wilkins, 1989). The combination of increased bone strength (Jendral *et al*., 2008) and improved muscle metabolic capabilities suggest that hens housed in FC could provide muscle that is more suitable for meat production. Stronger bones also suggest that FC hens contend with less bone fractures and associated pain than hens housed in CC. This demonstrates an important improvement to the health and well-being of hens housed in the FC housing system.

In both studies presented in this thesis, the results have consistently indicated that SH and LL hens exhibit muscle characteristics indicative of an increased stress response as compared to

LB hens. An increased hen stress response can ultimately yield undesirable meat properties such as tougher and redder meat. The strain differences presented in this research indicate the importance of selecting a suitable hen strain and housing system combination. It is important for a producer to both consider the animal's ability to cope within a specific environment and to provide a housing system in which environmental stress is kept to a minimum. For example, decreasing group size in FC could help reduce stress for all strains examined in this study and consequently improve meat quality. Suitable strain selection and reduced physiological stress would not only improve meat quality, but would enrich hen welfare as well.

4.3 Future Directions

4.3.1. Strain Differences

The strain differences in strong ion concentrations and consequent [SID] observed in this research (Chapter 3) seem to indicate that variations in ion transport capacity may have resulted from lower expression of the NCX transporter and the Na^+/K^+-pump but higher expression of MCT within SH and LL hens compared with LB hens. Previous research indicates that such an adaptive pattern is associated with stress related increases in glucocorticoids (Smith & Smith, 1994; Ravn & Dorup, 1997). Thus, it is recommended that future studies quantify NCX, Na^+/K^+-pump and MCT protein and mRNA expression levels, in order to investigate their relationship with rising glucocorticoids levels. A comparison of the relationships between glucocorticoid levels, ion transport capacity, and the $\sum([Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+])$ between hen strains should provide essential data that establish important mechanistic underpinnings that account for strain differences in post-mortem $[H^+]$. Such information may prove useful to establish group size recommendations based on genetic strain.

4.3.2. Housing Differences

To gain a better understanding of the differences between housing systems and between genetic strains observed in this research it is recommended that future studies evaluate muscle fibre types and conduct metabolic measures to determine if hens housed in the FC system undergo significant transformation from a fast, glycolytic phenotype to a slower, more oxidative phenotype that corresponds with improved bone strength (Jendral, 2012). Research presented in Chapter 3 indicates that greater strong base cation uptake and greater CO_2 production within *Pectoralis major* muscles of hens housed in the FC, compared with the CC housing systems, are consistent with a fast-to-slower fibre type transition. This hypothesis is further supported by Branciari *et al.* (2009) who showed that increased activity induced a fibre type shift from highly glycolytic to a slower, more oxidative phenotype within the *Pectoralis major* muscles of White Leghorn hens.

It was further hypothesized that the opportunity for greater activity within the FC system would generate stronger bones and healthier meat that is more resistant to both initial and ultimate post-mortem increases in $[H^+]$. Importantly, these adaptive changes should have yielded meat with greater commercial value. Because hens housed in the FC system also experienced stress in relation to group size, future meat quality studies should examine optimal group sizes for laying hen strains typically used for commercial egg production.

Overall, this research has presented a novel, biochemical approach to studying animal welfare. Animal welfare can be assessed through measures of an animal's physical condition, behaviour and productivity (Fraser, 2003). Laying hens housed in FC exhibit improved bone health (Jendral *et al*., 2008; Jendral, 2012), increased expression of highly motivated behaviours such as nesting, foraging and perching, and decreased expression of aggressive behaviours

(Jendral, 2012), all of which support that animal welfare was improved for hens housed in FC over hens housed in CC. In Chapter 2, we investigated meat quality and expected that since hens housed in FC are allowed increased space and the amenities to promote natural behavioural expression they would exhibit improved muscle health and meat quality over hens housed in CC. However, contrary to our expectations, there was an overall lack of differences in meat quality between CC and FC which suggested that hens housed in both systems experience stress. Nonetheless, when muscles were examined at a cellular level using the Stewart Model of acid-base regulation in Chapter 3, differences were revealed between CC and FC which suggested that the increased movement in FC benefitted hen muscle health. The Stewart model has been shown to identify changes in muscle cell physiology that standard meat quality measures could not and has therefore been identified as a new tool to assess animal welfare.

4.3.3. The Stewart Model of Acid-Base Regulation

The research presented in this thesis has shown that the Stewart Model is an outstanding tool for researchers to adopt in order to gain a better overall understanding of meat quality, not only for avian species, but for mammalian species as well. Due to the limitations of this research, only the *Pectoralis major* muscle samples that were taken at 17 min post-mortem were examined for the three systems-independent variables encapsulated in the Stewart Model. Since meat quality is affected by the physical and chemical changes occurring in the muscle beyond 17 min post-mortem, it is recommended that 24 h post-mortem *Pectoralis major* muscle samples be analyzed using the Stewart model as well. It is also recommended that future studies collect ante-mortem and post-mortem biopsies of the *Pectoralis major* in order to better gauge the contribution of glycogenolysis to lactate formation under production conditions. This would allow researchers to directly measure glycogen potential and to determine when Lac^-

accumulation occurs during processing. In the present research, direct measures of strong base cations and strong acid anions allowed us to accurately determine the contributions of the [SID] to variations in the prevailing [H^+]. Further, grouping the ions according to the $\sum([Ca^{2+}]+[Mg^{2+}]+[Na^+]+[K^+])$ and the $\sum([Cl^-]+[Lac^-])$ provided unique insights revealing the source of fluctuations in the [SID] and suggested considerable plasticity exists within this parameter. This finding could potentially be exploited by adding desirable amounts of strong base cations to hen diets that could serve to defend against post-mortem acidification of meat and optimize post-mortem [H^+].

Although A_{tot} did not significantly vary between hen strains or housing conditions, future studies could confirm that estimates of K_A reported for avian plasma, reported by Stämpfli *et al.* (2006) (*i.e.*, between 2.15 x 10^{-7} and 3.25 x 10^{-7} Eq/L, or pKA ~ 6.5 – 6.7), are that same in *Pectoralis major* muscles from various hen strains. This could be completed by applying CO_2 tonometry to whole muscle homogenates. A clearer understanding of the contribution of $A_{tot,}$ the most stable component of the Stewart model, to individual hen strains and between various production settings should allow researchers to increase or decrease A_{tot} as required to optimize post-mortem acidification. For example, increased dietary protein and glucose should elevate A_{tot} and [H^+] by increasing the intracellular concentrations of amino acids and phosphorylated glycolytic intermediates, respectively. In contrast, A_{tot} and [H^+] may be reduced by allowing laying hens greater opportunity for movement. Chronic movement could, for example, reduce the net expression levels of cytosolic proteins (*e.g.*, parvalbumin, glycolytic enzymes) and reduce the transient movement of plasma albumin into muscle cells. The importance of controlling this parameter may prove important in reducing the incidence of PSE meat.

The source of greater CO_2 in FC observed in the present study remains to be determined. To determine if greater CO_2 accumulation observed in FC occurred within the ante-mortem or post-mortem periods, biopsies should be taken before and after death. To further understand the source of CO_2 and it relationship to greater activity in FC, measures of carbonic anhydrase activity and/or expression also seem warranted, given that carbonic anhydrase (CA) is elevated at this stage. Confirmation of greater carbonic anhydrase expression in FC could form a feasible basis for slowing the rate of post-mortem acidification. For example, adding Na-HCO_3 to hen diets would result in muscle uptake followed by rapid dissociation into Na^+ + HCO_3^-, and carbonic anhydrase-dependent conversion to CO_2 and H_2O, with the former being removed via the lungs. The resultant accumulation of muscle Na^+ should, of course, elevate the [SID] and reduce the rate of post-mortem acidification.

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