Occasionally… what you have to do is go back to the beginning and see everything in a new way.

-Peter Straub

University of Alberta

On-Farm and *Ante Mortem* Factors Affecting Broiler Quality

by

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in
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DEDICATION

To John and Lorraine Schneider, for supporting the latest in a very long list of crazy ideas. I am honoured to call you mom and dad.

Anna, Sarah and Elijah. There was only one of you when I started this crazy scheme. I'll have more time to spoil you rotten now that this is finished. If I can do this, so can you.

ABSTRACT

Experiments were conducted to determine the effects of nutrition, temperature during feed withdrawal, shackling duration, sex and age at processing on broiler quality. Low energy (94% of recommended) diets resulted in a lower percentage of carcass fat while increasing the percentage of carcass protein. Low protein (85% of recommended) resulted in a decreased percentage of carcass protein while carcass fat increased. Low protein diets also limited frame size as measured by length and width of *P. major*. Exposure to 9 C temperatures during feed withdrawal resulted in improved meat quality as measured by higher ultimate pH, lower drip loss and darker color. Long shackling time (120 s) did not affect ultimate pH, drip or cooking losses compared to short shackling (<10 s); however, short shackled broilers exhibited poorer tenderness values. Males had higher carcass protein and lower fat than females. Females exhibited higher ultimate pH, higher drip loss and lighter breast meat. Drip loss and ultimate pH decreased with age. Processing age and sex of broilers may have greater influences on meat quality than previously reported.

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1. Introduction

1.1 QUALITY IS IN THE EYE OF THE BEHOLDER

Quality refers to a 'degree of excellence' or 'superiority in kind' (Merriam-Webster Online Dictionary, 2008). These definitions point to a central problem with defining the quality of an item – it is often subjective. The degree to which an attribute contributes to the overall perception of an item's quality will vary.

Three sets of characteristics have been identified to assess the quality of an item: search, experience and credence attributes (Nelson, 1970; Cho and Hooker, 2002). Search attributes are those quality traits (e.g. color, size) that can be fully examined without consumption of a product (Nelson, 1970; Steiner, 2006). Experience attributes (e.g. taste or tenderness) are those traits whose quality can be fully examined only via consumption of a product (Nelson, 1970). Finally, credence attributes are those traits (e.g. a product's adherence to a food safety program) whose quality cannot be fully examined even after consumption (Cho and Hooker, 2002; Steiner, 2006).

Search, experience and credence attributes may all be used to assess the quality of poultry products. Both meat processors and consumers have a vested interest in obtaining high quality meat products. It has been suggested that the poultry industry encompasses two quality models; namely, market quality and process control (Fletcher, 2004). Market quality may be defined by extrinsic attributes such as appearance, color, or intrinsic attributes such as texture, composition, production system (e.g. organic, free-range) or brand name (Wood, 1995; Aberle *et al.*, 2001; Barbut, 2002; Fletcher, 2004). Process control is more concerned with products meeting specific guidelines for size, shape, appearance, functional properties and uniformity (Fletcher, 2004).

The poultry meat industry in North America has evolved substantially over the past several decades. In 1962, 83% of broilers were marketed as whole carcasses; 15% as cut-up products and 2% as further processed products (National Chicken Council, 2008). Some further processed products include breaded, smoked, marinated and delicatessen items (Baéza, 2000). By 2008, it was estimated that 48% of broilers were marketed as further processed products; with 41% marketed as cut-up products and the remaining 11% as whole carcasses (National Chicken Council, 2008). This is a dramatic shift in market priorities and follows increasing consumer demand for products that are quick and easy to prepare (Kranen and van Esbroeck, 2005). The focus on further

processing has emphasized the importance of producing chicken with good functional properties (e.g. pH, water holding capacity (**WHC**)).

The structure and function of muscle in the living animal profoundly influences the quality of the muscle once it is converted to meat. External factors such as on-farm management practices and *ante mortem* handling can also affect meat functional properties (Table 1.1). The following sections will provide a basic description of muscle structure and contraction, the processes that govern the conversion of muscle, key indicators of poultry meat quality and conclude with a discussion of growth and nutritional effects on carcass and breast composition.

1.2 MUSCLE STRUCTURE & COMPOSITION

1.2.1 Structure

It would be difficult to discuss meat quality without a general understanding of the basic structural properties of muscle. In general, muscles have the same basic structure and act as independent units but the shape and purpose of individual muscles depends on their function (movement, support) and skeletal attachment (Davies, 2004).

A schematic diagram of muscle structure is presented in Figure 1.1. The basic structural unit of muscle is the muscle fibre (structure (c) in Figure 1.1) and is made up of highly specialized smaller units known as myofibrils (structure (d) in Figure 1.1) (Lawrence and Fowler, 1997; Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002). A single muscle fibre can be composed of 1-2000 regularly arranged myofibrils (Warriss, 2000) and is covered by a layer of connective tissue known as endomysium (Barbut, 2002). Muscle fibres are aggregated into bundles (structure (b) in Figure 1.1); muscle bundles are separated from each other by connective tissue known as the perimysium (see structure (a) in Figure 1.1) (Barbut, 2002). Numerous muscle bundles combine to form muscle (structure (a) in Figure 1.1); muscles are covered by connective tissue known as epimysium (Barbut, 2002).

Myofibrils are in turn composed of even smaller units known as myofilaments (structure (e) in Figure 1.1); there are two types of myofilaments, thick and thin (Lawrence and Fowler, 1997; Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002). The thick and thin myofilaments differ in dimension, chemical composition and position within the myofibril (Aberle *et al.*, 2001). Thin filaments are composed of actin and are anchored at one end in perpendicularly aligned Z-disks or lines (shown in structures (d) and (e), Figure 1.1) (Wick, 1999). Specialized Z filaments compose the Z disk and connect actin filaments on either side of the Z-disk (Aberle *et al.*, 2001). Thick filaments

(Figure 1.2) are composed of myosin; myosin molecules have an elongated rod shape, with a pair of pear-shaped lobes forming the head region (Warriss, 2000; Barbut, 2002). The myosin molecule has a tail region formed of two long strands wound in a helical arrangement (Warriss, 2000). Thick filaments (Figure 1.2) are an aggregate of myosin molecules with heads sticking out at regular intervals (Warriss, 2000). The myosin heads can split ATP into ADP and phosphate (Barbut, 2002). The arrangement of thick and thin filaments has been described as having a lattice-like structure (Millman, 1998).

The striated appearance of skeletal muscle results from the arrangement of the myofilaments within the sarcomere (shown in structures (d) and (e), Figure 1.1, Figure 1.3); the sarcomere is the fundamental contractile unit of striated muscle and is defined as the segment of the myofibril between two Z-disks (Wick, 1999). The sarcomere is divided into regions that are based on the type of myofilaments present in each area; the I band contains only thin filaments, the H zone contains only thick filaments, and the A band contains interdigitated thick and thin filaments (Lawrence and Fowler, 1997; Lodish *et al.*, 2000).

Being composed of thin filaments, the I band has a lighter appearance than the more dense A band which is composed of both thick and thin filaments (Aberle *et al.*, 2001). The I and A bands are bisected by thin dense bands; the I band is bisected by the Z disk while the A band is bisected by the H zone (Lawrence and Fowler, 1997; Aberle *et al.*, 2001). Finally, the space between the filaments is made up of sarcoplasm, a dilute solution of salts and other proteins (Huxley, 1969).

1.2.2 Composition

Depending on its type, muscle is composed of 65 to 80 % water, 1.5 to 13 % fat, 1.5 % non-protein nitrogenous substances, 0.5 to 1.5 % carbohydrates, 1% inorganic compounds and 16 to 22% protein (Aberle *et al.*, 2001).

1.2.2.1 *Muscle Proteins*

There are three types of protein located within muscle: sarcoplasmic, myofibrillar and stromal (Barbut, 2002). Sarcoplasmic proteins are water soluble and contain myoglobin and hemoglobin, responsible for oxygen storage and transport in muscle (Aberle *et al.*, 2001; Barbut, 2002). Myofibrillar proteins include contractile, regulatory and cytoskeletal proteins (Aberle *et al.*, 2001). Actin and myosin are the major contractile proteins; when they interact during contraction the resulting protein compound is known as actomyosin (Warriss, 2000). Tropomyosin and troponin are regulatory

proteins associated with the thin filaments (Wick, 1999; Aberle *et al.*, 2001; Barbut, 2002). Cytoskeletal proteins such as titin and nebulin are structural components of myofibrils (Aberle *et al.*, 2001). The stromal proteins collagen and elastin compose connective tissue (Davies, 2004).

1.2.2.2 *Connective tissue*

Muscle connective tissue is the medium through which nutrients and waste products pass between muscle fibres and capillaries; connective tissues also provide structural support to muscles (Davies, 2004). As mentioned previously, there are three types of connective tissue that may be classified based on their location in the muscle: epimysium, perimysium and endomysium (Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002; Davies, 2004).

As mentioned earlier, connective tissue is composed mainly of two proteins: elastin and collagen. Elastin has impressive elastic properties and can stretch 250% without permanent damage; it is present in perimysium, the connective tissue that surrounds muscle bundles (Davies, 2004). Collagen is relatively insoluble and has high tensile strength and is present in epimysium, perimysium and endomysium (Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002). Muscle collagen has a profound impact on meat production due to its distinctive properties (Davies, 2004). Polypeptide chains weave together to form collagen molecules, in turn the molecules link together to form collagen fibrils, which ultimately constitute collagen fibres (Davies, 2004). The overall structure of collagen has been described as quasi-crystalline (Lepetit, 2008). The various cross-links between molecules, fibrils and fibres give collagen its high tensile strength and insoluble properties (Aberle *et al.*, 2001; Davies, 2004).

As animals age, these cross-links increase in number and strength which ultimately contributes to decreased meat tenderness in older animals (Bailey, 1985; Aberle *et al.*, 2001; Davies, 2004; Lepetit, 2007; 2008). During cooking, collagen transforms from a quasi-crystalline arrangement to a random structure with rubber-like properties (Lepetit, 2008). The effect of this rearrangement on meat tenderness will be discussed in section 1.7.2.3.

1.2.2.3 *Muscle Fibres*

Muscle fibres can influence meat quality due to their size, density, biochemical and physiological characteristics (McKee, 2003). Muscles are often a mosaic of different fibre types (Taylor, 2004). Fibres can be classified into three categories: Type I, IIA and

IIB and have the following characteristics (Peter *et al.*, 1972; Aberle *et al.*, 2001; Barbut, 2002; McKee, 2003; Taylor, 2004):

a) Type I. These fibres are slow contracting, red in color (due to high myoglobin content) and utilize oxidative metabolism. Oxidative metabolism requires a steady supply of oxygen. Type I fibres tend to be involved in endurance activities such as maintaining posture.
b) Type IIA: These fibres are fast contracting and utilize glycolytic and oxidative metabolism. Myoglobin content is high in this fibre type; they are utilized when rapid activity is required and fatigue slowly. Glycolytic metabolism can operate in the presence or absence of oxygen (aerobically or anaerobically).
c) Type IIB: These fibres are fast contracting, white in color and utilize glycolytic metabolism. Myoglobin content in these muscles is low. Type IIB fibres are predominantly involved in activities that use glucose as fuel (e.g. sprinting). They are more easily fatigued than Type I or IIA fibres.

Poultry breast muscle has a higher number of type II fibres; Type II fibres have been associated with stress susceptibility and meat quality problems in swine (McKee, 2003). The rapid glycolytic capacity of these muscle fibres can lead to low pH of the muscle while carcass temperatures is high *post mortem*; this set of circumstances can result in a condition known as pale, soft and exudative (PSE) meat (Offer, 1991; McKee, 2003). *P. major* muscle in chickens is composed of approximately 95% type IIB fibres; these fibres are responsible for the white color, poor juiciness and bland flavour relative to other muscles (Taylor, 2004). Chicken leg muscles have an abundance of Type I fibres and tend to be relatively more flavourful, juicy, and tender than breast meat (Taylor, 2004).

1.3 MUSCLE CONTRACTION

Contraction is signaled by a electrical stimulus from the brain and is transmitted by nerve fibres to muscle cells (Barbut, 2002). The net result of the nerve impulse is the release of calcium ions from the sarcoplasmic reticulum into the sarcoplasm; the calcium ions then interact with the regulatory proteins of contraction, troponin and tropomyosin (Barbut, 2002). Troponin is actually composed of three subunits: troponin I, C and T (Jackson *et al.*, 1975). The troponin T subunit is bound to tropomyosin while troponin I inhibits the interaction between actin and myosin (Jackson *et al.*, 1975). Troponin C preferentially binds with calcium ions released from the sarcoplasmic reticulum; when calcium binds to the C subunits the structure of troponin is altered so that myosin binding

sites on actin are exposed (Jackson *et al.*, 1975; Aberle *et al.*, 2001). Muscle contraction occurs in a repeating cyclic fashion; cross-bridges form as myosin heads attach to actin, pulling the thin filaments toward the center of the sarcomere and detaching (Goldman and Huxley, 1994). The hydrolysis of ATP to ADP provides the energy for this movement (Davies, 2004). The presence of sufficient ATP results in relaxation of muscle by dissociating actin and myosin (Davies, 2004). Muscle remains in a relaxed state as long as calcium is located in the sarcoplasmic reticulum and a high concentration of ATP is present in the cell (Aberle *et al.*, 2001; Barbut, 2002). ATP is a product of energy metabolism, which will be discussed in the next section.

1.4 MUSCLE ENERGY METABOLISM

In living muscle, ATP production is fueled by free fatty acids or glucose in the blood or from glycogen stored within the muscle (Warriss, 2000). Glycogen is mobilized when fatty acids and glucose are not broken down quickly enough to provide energy for the muscle cells (Warriss, 2000). ATP is produced when glucose or glycogen are broken down (Aberle *et al.*, 2001). Glycolysis converts glucose or glycogen to 2 molecules of pyruvic acid, 4 hydrogen ions and either 2 or 3 ATP molecules depending on the starting material (Aberle *et al.*, 2001). Glucose requires an ATP molecule to be broken down and results in 2 ATP molecules; the phosphorylation of glycogen results in the production of an extra ATP molecule (Warriss, 2000; Aberle *et al.*, 2001). Pyruvic acid reacts further via the tricarboxylic acid (TCA or Krebs) cycle under aerobic conditions to yield 36 ATP molecules from a single molecule of glucose (Honikel, 2004b). Under anaerobic conditions, the hydrogen ions released during glycolysis and the TCA cycle cannot combine with oxygen and subsequently accumulate in the muscle (Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002). The excess hydrogen ions are then used to reduce pyruvic acid to lactic acid so metabolism can continue (Aberle *et al.*, 2001). If anaerobic conditions persist lactic acid continues to build up in the muscle, ultimately resulting in a lower muscle pH (Warriss, 2000). As pH approaches 6.0 to 6.5, the rate of glycolysis is compromised (Aberle *et al.*, 2001). If oxygen reenters the system, lactic acid can be transported from the muscle and either converted back to glucose or metabolized to carbon dioxide and water in the heart (Aberle *et al.*, 2001). After death the anaerobic glycolytic metabolic pathway will be utilized exclusively; this process dominates the conversion of muscle to meat.

1.5 CONVERSION OF MUSCLE TO MEAT

The process by which muscle is converted to meat is a multi-step process originating with the slaughter of an animal and is the result of muscle acidification *post mortem*. The pattern and rate of acidification can affect color, water holding capacity and texture qualities of cooked meat (Warriss *et al.*, 1999).

After death oxygen is quickly depleted. As described previously for living muscle, anaerobic metabolism prevails and is marked by an increase in lactic acid with a corresponding decrease in muscle pH. However, pH will not decrease indefinitely; as pH decreases enzyme systems that promote lactic acid production are inhibited and eventually cannot function (Warriss, 2000; Barbut, 2002). Once lactic acid production ceases pH stabilizes at a point known as ultimate pH (Warriss, 2000). Decreases in pH reduce repulsive forces between myofilaments allowing the filaments to move closer together (Diesbourg *et al.*, 1988; Millman, 1998; Swatland, 2008). During acidification denaturation of sarcoplasmic proteins and shrinkage of myofibrils increases the light scattering properties of the muscle by; increased light scattering results in lighter meat (Offer *et al.*, 1989; Alvarado and Sams, 2000).

The acidification of muscle and depletion of ATP *post mortem* triggers the onset of *rigor mortis*. Once the supply of ATP falls below ~5 mmol per kg (Warriss, 2000) actin and myosin filaments permanently bind to each other and form actomyosin cross-bridges (Warriss, 2000; Aberle *et al.*, 2001; Davies, 2004). Actomyosin cross-bridges form gradually throughout a muscle; as each cross-bridge is formed a short movement of *rigor* shortening occurs (Honikel, 2004a). As more muscle fibres enter *rigor* carcasses become progressively more stiff (Warriss, 2000).

Muscles do not remain stiff indefinitely. Calpains are the main proteolytic enzymes responsible for catabolism and recycling of protein in living muscle; *post mortem* these enzymes are responsible for the softening process known as tenderization (Warriss, 2000). Normally, calpastatin binds to calpains to inhibit proteolytic activity (Dransfield, 1993). The presence of calcium ions breaks the bond between calpains and calpastatin; ultimately calpains degrade structural components at the Z disks (Dransfield, 1993; Dransfield and Sosnicki, 1999). Flexibility does not drop to pre-*rigor* levels as the result of tenderization (Aberle *et al.*, 2001; Barbut, 2002). The proteolytic process completes the conversion from muscle to meat (Warriss, 2000).

1.6 FUNCTIONAL PROPERTIES OF MEAT

1.6.1 pH

The conversion of muscle to meat may be thought of conceptually as a cascade of events triggered by the acidification of muscle *post mortem*. Ultimate pH and the rate of pH decline have implications for meat quality and color development (Barbut, 2002). A decrease in pH by 1 pH unit have been reported to increase protein denaturation by as much as 12 times (Offer, 1991). As protein integrity degrades its ability to bind water decreases (Warriss, 2000). In addition, once pH reaches the isoelectric point for myosin and actin the number of positively and negatively charged groups on these molecules are equal and will lose water normally bound to them (Warriss, 2000; Aberle *et al.*, 2001). Deviations from normal acidification rates can seriously compromise the ability of muscle protein to bind water. Three *post mortem* acidification patterns are illustrated in Figure 1.4 (Barbut, 2002). Each scenario has implications for the functional properties of meat. The middle line shows a normal ultimate pH and rate of pH decline; meat functional properties in this scenario are acceptable. The top line (Figure 1.4) represents a dark, firm and dry (DFD) meat type; limited glycogen energy reserves at slaughter subsequently result in decreased lactic acid production and high ultimate pH (Aberle *et al.*, 2001; Barbut, 2002). When pH is high *post mortem* protein groups remain tightly bound to each other and muscle water tends to be held intracellularly (Warriss, 2000; Aberle *et al.*, 2001; Barbut, 2002). Furthermore, high ultimate pH limits protein denaturation, which also influences the amount of intracellular water (Warriss, 2000; Aberle *et al.*, 2001). Meat appears darker since water is held intracellularly and is not available to reflect light as in the PSE scenario (Warriss, 2000).

The bottom line (Figure 1.4) represents the PSE meat type. Typically this scenario results from stress immediately prior to slaughter. Lactic acid buildup immediately post slaughter is higher than normal, resulting in a low muscle pH while carcass temperature remains high (Offer, 1991; Alvarado and Owens, 2008). The combination of low pH and high temperature accelerates protein denaturation which decreases the ability of muscle protein to bind water (Briskey, 1964; Warriss, 2000; Sams and Alvarado, 2004). There is also evidence that suggests that myosin shrinkage under PSE conditions draws thick and thin filaments closer together, thereby expelling water from the muscle (Diesbourg *et al.*, 1988; Offer, 1991). In the PSE scenario color is affected since large amounts of water are held extracellularly; water has a high capacity

for reflecting light which makes the meat appear pale (Warriss, 2000; Aberle *et al.*, 2001).

The biochemical mechanisms that cause the PSE condition are poorly understood (Olivo *et al.*, 2001). However, recent research has indicated that increased formation of reactive oxygen species (**ROS**) associated with oxidative stress may play a significant role in the development of this syndrome (Betti *et al.*, 2009; Wang *et al.*, 2009). ROS are formed as a result of several metabolic processes, including hypoxia and anoxia in skeletal muscle (Chance *et al.*, 1979; Sohal and Weindruch, 1996; Clanton, 2007). Oxidative damage occurs under conditions of oxidative stress, that is, excessive ROS production or a decrease in protective antioxidants (Sohal and Weindruch, 1996; Grimsrud *et al.*, 2008). If a cell is undergoing oxidative stress, cellular constituents are oxidized such that DNA, proteins, lipids and carbohydrates may be modified (Grimsrud *et al.*, 2008). ROS are not all bad – they may be involved in cellular regulation that mediates the effects of oxidative stress; however, in high concentrations ROS are hazardous and have damaging effects on cellular components (Droge, 2002). During *post mortem* metabolism, when hypoxia and anoxia take place, the natural defenses of muscle cells fail and ROS can increase to levels that negatively affect the conversion of muscle to meat.

With respect to the formation of PSE meat, ROS have been implicated in faster rates of glycolysis and lactic acid production via their role in the activation of adenosine monophosphate activated protein kinase **(AMPK)** (Betti *et al.*, 2009). AMPK increases the supply of cellular energy by switching on ATP-generating pathways and decreasing energy demand by switching off ATP-utilizing pathways (Proszkowiec-Weglarz *et al.*, 2006; Richter and Ruderman, 2009). Heat stress has been linked to increased levels of ROS (Mujahid *et al.*, 2006). Wang *et al.* (2009) observed that exposure to heat stress conditions (40°C) for 1 to 5 hours resulted in greater oxidative damage to sarcoplasmic and myofibrillar protein. Betti *et al.* (2009) observed a profound reduction in ultimate pH and functional properties of meat when feeding flaxseed and theorized that the formation of ROS as a consequence of n-3 polyunsaturated fatty acid deposition was responsible. Interestingly, the effects of ROS in the formation of PSE meat have been mitigated by the inclusion of dietary antioxidants (Shen and Du, 2005; Shen *et al.*, 2005). Some researchers have proposed marination methods to remediate the effects of the PSE-like condition in turkeys (Alvarado and Sams, 2003).

Pre-slaughter stress such as struggling behavior or excessive heat can accelerate *rigor* development (Northcutt *et al.*, 1994; McKee and Sams, 1997), muscle acidification *post mortem* and can lead to pale meat and poor WHC (Papinaho *et al.*, 1995; Owens and Sams, 2000; Aberle *et al.*, 2001; Sandercock *et al.*, 2001; Berri *et al.*, 2005; Debut *et al.*, 2005; Barbut *et al.*, 2008).

It has been suggested that diet has limited influence on the functional properties of meat, with the assumption that feeding practices do not alter the amount of muscle glycogen (Edwards *et al.*, 1999; Aberle *et al.*, 2001). However, recent research has indicated that increasing the amount of dietary lysine can modify *P. major* ultimate pH (Berri *et al.*, 2008). The metabolic reasons for this are unclear, but the mechanism is theorized to be decreased glycogen storage within muscle and a subsequent reduction in acidification *post mortem* (Berri *et al.*, 2008). Betti *et al.* (2009) reported negative effects on *P. major* functional properties as a consequence of feeding flaxseed.

Satterlee *et al.* (2000) reported that males were more likely to exhibit struggling behavior during shackling. However, several authors have reported no differences in muscle pH attributable to sex (Quentin *et al.*, 2003; Cavitt *et al.*, 2005). Finally, pH has been reported to decrease with bird age (Sandercock *et al.*, 2001).

Strong correlations between muscle pH and color have been reported (Fletcher, 1999a). In light of the significant influence of pH on color, this relationship is often suggested as a tool to predict meat quality in broilers (Barbut, 1993; Fletcher, 1999a).

1.6.2 Water Holding Capacity

Water holding capacity (WHC) has been defined as 'the ability of meat to hold fast its own or added water during application of any force' (Pearson, 1986). Poultry products typically contain 60-80% moisture which is largely contained in the protein matrix within the muscles (Barbut, 2002). Water held within muscle has three distinct categories as described by Aberle *et al.* (2001), Barbut (2002), Huff-Lonergan and Lonergan (2005) and Kolczak *et al*. (2007):

a) Bound. Water polarity results in associations or binding with reactive groups of amino acid side chains. This type comprises 4-10% of total water in the muscle and will remain tightly bound despite strong physical forces.
b) Immobilized. Water molecules attracted to bound water in successive layers. These bonds become progressively weaker as distance from the charged amino acid side chains increases. The amount of immobilized water can vary depending

on net charge of the proteins, structure of the muscle cell and extracellular space within the muscle.

c) Free. Weak surface forces retain this water in the muscle. It can be easily removed and is the type that flows from meat during pressing or centrifugation. If moisture is added to the meat during processing, it joins this category of muscle water and is therefore of great significance to the further processing industry.

There are three main factors that affect the ability of muscle proteins to hold water as a result of the decrease in pH after death (Barbut, 2002).

a) Net charge effects: The decrease in pH after slaughter decreases the ability of protein to bind water, either through denaturation or changes to the net charges of protein groups (Warriss, 2000; Aberle *et al.*, 2001).
b) Steric effects: Cross-bridge formation between actin and myosin is responsible for *rigor mortis,* but also decreases WHC by reducing space for water to dwell within the muscle (Offer and Trinick, 1983; Huff-Lonergan and Lonergan, 2005).
c) Ion exchange: Following *rigor mortis* resolution, cellular structures are degraded through enzyme activity and ions are redistributed (Barbut, 2002). Divalent calcium ions bound to protein groups can be replaced by monvalent ions like sodium, freeing up space to bind additional water (Barbut, 2002).

Several methods have been proposed to measure the WHC of meat. Methods to measure WHC can be divided into two main categories: gravity or pressure application (Warriss, 2000; Barbut, 2002). Gravity methods are typically the simplest and involve storage of samples for a specified amount of time; water lost by the muscle is measured to provide an indication of WHC (Warriss, 2000). Thermal force (cooking) is another technique used to determine WHC (Barbut, 2002). Centrifugation has been used to measure exudate from meat samples; it has also been used in conjunction with salt solutions to measure the uptake of additional moisture by meat (Barbut, 2002).

Pre-slaughter stress affects muscle acidification *post mortem* and can significantly affect WHC (Owens and Sams, 2000; Aberle *et al.*, 2001; Sandercock *et al.*, 2001; Debut *et al.*, 2003; Berri *et al.*, 2005; Debut *et al.*, 2005). Other factors such as diet, sex of bird and age may also affect WHC. Feeding practices that alter the amount of glycogen have the greatest impact on WHC (Aberle *et al.*, 2001). Berri *et al.* (2008) reported that increasing dietary lysine from 0.83 to 1.13% of the total finisher diet resulted in higher ultimate pH and improved WHC in Ross 308 broilers, possibly due to

decreased glycogen storage within muscle. Feeding flaxseed has been linked to the development of PSE meat with decreased WHC (Betti *et al.*, 2009). Another study modified total dietary crude protein and did not observe any effect of dietary protein concentration on WHC; however, this study utilized broilers of fast and slow growing genotypes and slaughtered at later ages, which could account for the differences (Quentin *et al.*, 2003).

Some studies have observed sex related differences in WHC; Lyon *et al.* (1983) observed that females had higher cook losses than males. Others have reported poor WHC in females compared to males (Fanatico *et al.*, 2005). Variable age related effects on WHC have been reported. No differences in drip losses between broilers aged 35 or 63 d were reported by Sandercock (2001). Cook losses have also been reported to decrease with bird age (Lyon *et al.*, 1983).

1.7 MEAT COLOR

Poultry meat color is influenced by several factors, including the heme pigments present in the meat (Froning *et al.*, 1968; Fleming *et al.*, 1991; Cornforth, 1994; Froning, 1995; Warriss, 2000; Barbut, 2002), age, strain, sex of bird (Froning *et al.*, 1968; Ngoka *et al.*, 1982), diet (Froning *et al.*, 1969), and preslaughter handling (Froning *et al.*, 1978; Babji *et al.*, 1982; Ngoka and Froning, 1982; Ngoka *et al.*, 1982; Fletcher, 1999b; 2002). The color of meat has a large influence on meat purchasing behavior since many consumers view discoloration as an indicator of freshness and overall quality (Barbut, 2002; Fletcher, 2002; Mancini and Hunt, 2005). Meat color is also an indicator of quality attributes that are not visible to the average consumer. As noted earlier in this review, pH influences the water-binding nature of muscle proteins and light reflectance properties of the meat (Briskey, 1964; Fletcher, 1999b).

Meat color is predominantly influenced by the presence of hemoglobin and myoglobin in the muscle (Warriss, 2000; Barbut, 2002). Myoglobin has two main parts, a protein component called globin and non-protein portion known as the heme ring (Barbut, 2002). At the center of the heme ring is an iron molecule that can form 6 bonds, 5 of which are nitrogen or histidine (Mancini and Hunt, 2005). The state of the remaining bond determines the form and color of the myoglobin (Barbut, 2002; Mancini and Hunt, 2005). Myoglobin has three forms:

a) Oxymyoglobin, bright red in appearance, heme iron is ferrous (Fe^{2+}), oxygen bound to iron molecule, MbO_2

b) Deoxymyoglobin, purplish-red, heme iron is ferrous (Fe^{2+}), no oxygen molecule bound to iron molecule, Mb

c) Metmyoglobin, brown, heme iron is ferric (Fe^{3+}), MetMb (Conforth, 1994; Mancini and Hunt, 2005)

The three forms refer to the state of the iron molecule in the center of the myoglobin complex. Myoglobin is typically found in highest concentrations in muscle cells where the primary function is to store oxygen until it is required by the mitochondria (Conforth, 1994). Changes between the three forms of myoglobin are reversible under normal conditions and occur as the result of oxygenation or deoxygenation (Barbut, 2002).

Muscle type and function have a strong influence on the amount of myoglobin present and subsequent effects on meat color (Nishida and Nishida, 1985). Slow-contracting red muscle has higher levels type I fibres, which have higher levels of myoglobin to provide for their largely aerobic energy metabolism; white muscle is composed largely of fast-contracting type II fibres that utilize anaerobic energy metabolism (Burke *et al.*, 1971; Peter *et al.*, 1972; Nishida and Nishida, 1985). Broiler breast muscle is largely composed of white muscle fibres and routinely utilizes anaerobic metabolism (Smith and Fletcher, 1988; Taylor, 2004).

1.7.1 Factors affecting poultry meat color

Bird age has been shown to influence *P. major* color in turkeys, with lightness values decreasing (Froning *et al.*, 1968; Ngoka *et al.*, 1982). In turkeys this change has been attributed to changes in heme pigment concentration (Froning *et al.*, 1968). Other authors have indicated that myoglobin concentration increases with age in most species which could account for the darkening effect (Froning *et al.*, 1968; Fletcher, 1999b; Warriss, 2000).

Sex of bird effects on meat color has not been reported in broiler chickens, however, Froning *et al*. (1968) observed that female turkeys tended to have lighter meat than males. Ngoka *et al* (1982) reported no color differences between male and female turkeys; the experiments used different color measurement methodologies which could explain the differences.

Dietary influences on broiler *P. major* color are attributed to quality or type of feed ingredients or level of carotenoids (Froning, 1995). The presence of nitrates in the diet results in redder meat (Froning *et al.*, 1969); the presence of mold cultures in diets has resulted in redder turkey meat (Wu *et al.*, 1994). Corn-based diets, higher in carotenoids than wheat, have been shown to result in yellower *P. major* (Lyon *et al.*,

2004). Berri *et al.* (2008) found that increasing dietary lysine resulted in darker colored meat in addition to the effects on higher ultimate pH and improved WHC.

Preslaughter handling can affect meat color. Broilers and turkeys exposed to cooler holding conditions (4 to 7°C) prior to slaughter tended to exhibit darker meat color along with higher ultimate pH (Froning *et al.*, 1978; Babji *et al.*, 1982; Dadgar *et al.*, 2008).

Exposure to cool temperatures results in mammals and birds generating heat by one of two processes– shivering or non-shivering thermogenesis (Hocquette *et al.*, 1998). Shivering is an involuntary contraction of myofibrils that utilizes ATP and increases the rate of energy substrate oxidation (Hocquette *et al.*, 1998). Reduced availability of energy substrates at slaughter after cold exposure likely limits lactic acid production, resulting in higher pH_u and darker muscle. Struggling prior to or during slaughter has been shown to result in redder meat (Froning *et al.*, 1978; Ngoka and Froning, 1982).

1.7.2 Meat Tenderness

1.7.2.1 *Tenderization*

As described previously, the amount of glycogen present at slaughter will affect the amount of ATP in the muscle. If glycogen is limited, ultimately ATP levels in muscle will decrease quickly, which will put muscles into *rigor* early (Warriss, 2000). At *rigor* the extent of muscle contraction will determine the tenderness of cooked meat (Aberle *et al.*, 2001). Tenderization does not result from actomyosin breakdown, but is caused by proteolytic degradation of myofibrillar proteins (Warriss, 2000; Aberle *et al.*, 2001). As described in section 1.5, calpains are the most important enzyme involved in proteolytic activity that tenderizes meat.

1.7.2.2 *Measurement of meat tenderness*

Two measurement methods are commonly used to measure tenderness of chicken meat, namely, Warner-Bratzler and Allo-Kramer shear. These methods measure the amount of force required to cut through muscle fibres which simulates the force required to chew (Lyon and Lyon, 1991). Sensory evaluations are another method used to measure meat tenderness, among other attributes (Barbut, 2002). A sensory evaluation has been defined by Barbut (2002) as ‘a scientific method used to evoke, measure, analyze and interpret those responses to products, as perceived through the sense of taste, touch, smell, sight and hearing.’ A combination of these measurement methods should provide a reasonable indication of chicken meat tenderness. In cases where sensory evaluation is not feasible, research has shown that the correlation coefficients between

objective measurements such as Warner-Bratzler or Allo-Kramer shear and sensory evaluations of tenderness are over 0.82 (Lyon and Lyon, 1990; Awonorin and Ayoade, 1992). Based on that research, it is reasonable to use objective measures as a approximation measurement of meat tenderness. However, as will be shown in the next section, the overall inconsistency in *P. major* tenderness is difficult to explain. Some have theorized that this inconsistency may be attributable to differences in measurement methods or the variability of shear values between birds (Poole *et al.*, 1999b); others have surmised that different rates of *rigor mortis* development are responsible (Cooper and Fletcher, 1997).

1.7.2.3 *Factors affecting meat tenderness*

Collagen cross-linking and insolubility increases with animal age which decreases meat tenderness (Bailey, 1985; Aberle *et al.*, 2001; Fletcher, 2002; Davies, 2004; Fanatico *et al.*, 2005; Lepetit, 2007; 2008). Cooking results in the transformation of collagen from a quasi-crystalline structure to a more random structure with rubber-like properties (Lepetit, 2008). These newly acquired rubber-like properties allow collagen to shrink to as little as 20% of its original length if unrestrained (Flory and Spurr, 1961; Kurth, 1993). Collagen cross-links act as restraining factors in cooked meat; collagen shrinkage generates tension that forces fibre bundles together and drives out moisture from the meat (Kurth, 1993; Lepetit, 2008). As the number of cross-links increases, the amount of force generated by collagen shrinkage will also increase; collagen shrinkage expels water from the muscle (Kurth, 1993; Lepetit, 2008). Tenderness is reduced because the meat is drier (Kurth, 1993; Betti *et al.*, 2009). Kurth (1993) also suggested that forcing the fibre bundles together increases the number of fibres per cross-sectional area; the force required to shear a given area by chewing is also increased. Several studies have investigated the influence of bird age on *P. major* tenderness with conflicting results.

Several studies that measure tenderness in poultry at various ages between 5 to 16 wk have observed decreases in tenderness as bird age increased (Awonorin and Ayoade, 1992; Poole *et al.*, 1999b; Northcutt *et al.*, 2001).

Tenderness differences between the sexes are variable. Work by Lyon *et al.* (1983) detected sex related differences in springiness and chewiness. Some studies have observed lower shear values in males compared to females (Simpson and Goodwin, 1975; Evans *et al.*, 1976). Conversely, Northcutt *et al* (2001) observed lower shear values in female broilers at 46 d of age. Several studies have indicated no differences in

tenderness measures between the sexes (Gilpin *et al.*, 1960; Shrimpton and Miller, 1960; Carlson *et al.*, 1962; Goodwin *et al.*, 1969; Farr *et al.*, 1983; Lyon and Wilson, 1986; Cavitt *et al.*, 2005).

Poole *et al.* (1999b) evaluated the effect of small differences in dietary crude protein content and 100 kcal/kg ME content on meat tenderness and observed no effects on Allo-Kramer or Warner-Bratzler shear values. Several studies have indicated that no differences in *P. major* tenderness were observed when dietary energy was modified (Goodwin *et al.*, 1969; Arafa *et al.*, 1985; Sonaiya *et al.*, 1990). Some evidence suggests that dietary ingredients can affect meat tenderness: corn fed broiler meat was more tender than wheat-fed meat (Lyon *et al.*, 2004).

Pre-slaughter handling factors have been linked with changes to meat tenderness. High rates of *post mortem* glycolysis and lactic acid production in conjunction with high carcass temperatures has been shown to deactivate the calpain system; since calpains are responsible for the *post mortem* proteolytic softening of post *rigor* meat a decrease in their activity results in tougher meat (Dransfield, 1994; Dransfield and Sosnicki, 1999). Wood and Richards (1975) observed that cool temperatures resulted in higher shear values, whereas Froning et al. (1978) observed lower shear values in cool treated birds compared to heat stressed and control birds. Free struggle during shackling has been shown to result in higher shear values (Froning *et al.*, 1978; Ngoka *et al.*, 1982).

1.8 NUTRITIONAL, AGE AND SEX EFFECTS ON CARCASS AND BREAST COMPOSITION

1.8.1 Growth

Growth may be described most simply as the process of getting bigger; changes in form are a response to changing physiological needs as an animal matures (Lawrence and Fowler, 1997). Growth is an incredibly complex process; it is dependent on genetics, nutrition and a host of environmental factors (Lippens, 2003). Furthermore, the biochemical processes involved in growth require time, as such, animals do not grow instantly (Lawrence and Fowler, 1997). The growth of a broiler chicken follows a sigmoid or S shaped pattern when weight of the bird is plotted against time; growth rates change throughout development from an accelerating phase post hatch to a maximum growth rate, followed by a decelerating phase (Lawrence and Fowler, 1997; Lippens, 2003). Components of the body have their own growth rates in response to physiological needs and as such the nutritional demands for specific tissues and organs will vary over time (Kwakkel *et al.*, 1993).

1.8.2 Diet composition and allocation

At the most general level, broiler diets are composed of carbohydrates, protein, fat, minerals and vitamins that provide the energy and nutrients required for growth, reproduction and health (National Research Council, 1994; Leeson and Summers, 2001). The nutrients are typically provided by a mixture of cereal grains, soybean meal, animal by-product meals, fats and vitamin and mineral premixes (National Research Council, 1994). The energy for metabolism and meat production in the broiler is primarily supplied by dietary carbohydrate and fat; however, in some cases dietary protein may be utilized (National Research Council, 1994). Broilers grow by depositing dietary nutrients in body tissues, mainly as fat and protein (Lopez *et al.*, 2007). Accretion of fat and protein in broilers can be affected by nutrition, genetics, sex, environmental conditions, body weight, degree of maturity or the interactions between these factors (Lopez *et al.*, 2007). Hammond (1944) suggested a framework for allocation of nutrients that was later adapted by Humphrey and Klasing (2004) which is reprinted in Figure 1.5. The brain and central nervous system have the highest priority, followed by bone, muscle and finally adipose or fat tissue (Humphrey and Klasing, 2004). Kwakkel *et al* (1993) characterized body weight growth to 32 wk in leghorn pullets as multiphasic; that is, growth occurs in four distinct phases. The first 2 phases to 14 wk were attributed to general growth, or the development of the skeletal system, feathers, muscle and maintenance organs like the digestive tract (Kwakkel *et al.*, 1993). The third phase of growth was associated with growth of the reproductive organs and the fourth phase was related to an increase in body fat (Kwakkel *et al.*, 1993).

1.8.3 Dietary Energy

Metabolizable energy (**ME**) is a standard measure used to describe poultry energy requirements or energy levels of a diet (National Research Council, 1994; Lopez *et al.*, 2007). ME is defined as the difference between gross energy of a feed and the gross energy of feces resulting from that feed (National Research Council, 1994). Broilers and turkeys adapt to variations in dietary ME by modifying their feed intake - assuming adequate amounts of other essential nutrients are provided in the diet (Hill and Dansky, 1950; 1954; Carlson *et al.*, 1962; Velu and Baker, 1974; National Research Council, 1994; Leeson *et al.*, 1996; Leeson and Summers, 2001). The selection of a dietary ME level that minimizes feed costs while maximizing gains is a primary decision when formulating broiler chicken rations, other nutrient levels are established based on that initial decision (National Research Council, 1994). It has been suggested that high

dietary energy concentrations will result in higher energy intake despite the broilers eating to their energy requirements (Leeson and Summers, 2001). This was illustrated by Jackson *et al* (1982), whose study reported increases in energy intake from 9,862 to 12,604 kcal as dietary ME levels increased from 2600 to 3600 kcal ME/kg in 49 d broilers. It has been suggested that since adjustments in feed intake in response to dietary energy level are not sufficient to keep energy intake constant, growth responses should be considered a function of dietary energy level (Gonzalez-A. and Pesti, 1993).

This theory has not been universally accepted. Emmans (1981; 1989) theorized that feed intake is determined by birds attempting to fulfill their genetic potential for growth rate; under this scenario birds increase their intake of a given feed to achieve that growth rate. In an experiment that limited the amount of dietary isoleucine, broilers increased their overall feed intake to make up for the nutrient deficiency (Burnham *et al.*, 1992). There is evidence that this theory of feed intake is not appropriate for all broiler strains; the theory describes the feed intake of Cobb 500 broilers but the Ross 308 ((Berhe and Gous, 2005; Kemp *et al.*, 2005) as cited by (Gous, 2007)).

A practical solution to this problem has been to increase the amount of other nutrients as dietary energy is increased. Once requirements for growth and maintenance have been met, excess dietary energy is stored as fat (Lin, 1981). Many researchers have reported that higher energy diets result in higher fat deposition in the carcass (or low energy diets result in lower fat deposition) (Hill and Dansky, 1954; Evans *et al.*, 1976; Pfaff and Austic, 1976; Jackson *et al.*, 1982; Campbell *et al.*, 1988; Boekholt *et al.*, 1994; Wiseman and Lewis, 1998). Carlson *et al*. (1962) did not observe any effects of dietary energy level on breast muscle composition of turkeys; since breast muscle typically does not have a high level of fat this is not surprising.

A study of 49 d old broilers conducted by Jackson et al. (1982) indicated that although energy intake increased as dietary energy level increased, protein intake decreased. Subsequent chemical analysis indicated that the high energy diets resulted in more carcass fat while leading to lower carcass protein and moisture (Jackson *et al.*, 1982). Research relating to carcass protein levels as a result of dietary energy levels have had mixed results – some studies have found that carcass protein was decreased as dietary energy increased (Summers *et al.*, 1965; Velu and Baker, 1974) while others did not detect any differences in carcass protein as a result of modifying dietary energy level (Jackson *et al.*, 1982). The differences in the age and type of bird utilized in each study are likely responsible for the discrepancy in conclusions. The Velu and Baker (1974)

study only investigated broilers to 21 d; the Summers *et al* (1965) investigated leghorn chickens to 42 d.

1.8.4 Dietary Protein

The National Research Council (1994) states that protein requirements are really requirements for the amino acids in the dietary protein. Amino acids are the building blocks for skin, feathers, bone, ligaments, organs and muscles (National Research Council, 1994). Sufficient amino acid intake is required to maintain the cycle of continuous protein accretion and degradation in the body (National Research Council, 1994). Animals utilize the same 22 amino acids as plants (Leeson and Summers, 2001). Essential amino acids are those that cannot be synthesized by animals or birds (methionine, arginine, tryptophan, threonine, histidine, isoleucine, leucine, lysine, valine and phenylalanine) and must be supplied in the diet (Leeson and Summers, 2001). Glycine, serine and proline are considered semi-essential amino acids; although they can be synthesized by chickens the rate of synthesis is not fast enough to maximize growth (Coon, 2002). Dietary balanced protein uses lysine as a reference amino acid (lysine is maintained at a constant proportion of crude protein) while minimum contents of all other essential amino acids are kept constant (Eits *et al.*, 2005). This is known as dietary balanced protein (DBP). Lysine constitutes 7% of the protein in breast muscle (*P. major* and *P. minor*) (Munks *et al.*, 1945; Dozier *et al.*, 2008) and therefore has great significance in poultry diets; insufficient dietary lysine affects breast muscle more than other muscles (Tesseraud *et al.*, 1996). Protein accretion requires that all necessary amino acids are available (Coon, 2002); therefore limiting lysine or other amino acids will result in reduced protein deposition (Dozier *et al.*, 2008). Increased breast yield as a result of increased dietary protein is believed to be a consequence of increased myofibre size (Allen *et al.*, 1979).

Excess protein or amino acids beyond metabolic requirements for protein synthesis are degraded as follows: nitrogen is disposed of in uric acid, while carbon skeletons of individual amino acids may be degraded for a) glucose synthesis, b) fat synthesis or c) directly to CO_2 + H_2O and energy (Leeson and Summers, 2001). Limiting dietary protein will reduce or stop growth; in such instances protein may be mobilized from body tissues such as muscle to provide carbon skeletons for glucose synthesis (National Research Council, 1994; Leeson and Summers, 2001).

Decreasing dietary protein below the levels recommended by the NRC (1994) has been shown to reduce meat yield and increase carcass fatness (Moran *et al.*, 1992).

Several researchers have demonstrated that increasing protein content in broiler diets results in leaner carcasses (Fraps, 1943; Summers *et al.*, 1965; Bartov *et al.*, 1974; Pfaff and Austic, 1976; Sibbald and Wolynetz, 1986; Cabel and Waldroup, 1991; Smith and Pesti, 1998; Smith *et al.*, 1998; Bregendahl *et al.*, 2002). Although carcass protein increases as a proportion of the carcass when dietary protein is increased, typically no effect is found on grams of protein or muscle in the carcass (Leeson and Summers, 2001).

1.8.5 Age effect on composition

Growth may be described most simply as the process of hypertrophy; changes in form are a response to changing physiological needs as an animal matures (Lawrence and Fowler, 1997).

The first week of life constitutes 17% of the life of a broiler marketed at 42 d of age. This encompasses the transition between embryonic yolk absorption to food (Nitsan *et al.*, 1991). The digestive system is a supply organ, that is, it supports the growth and maintenance of the rest of the body; supply organs such as the digestive system mature rapidly creating the foundation for the future growth of the rest of the body (Katanbaf *et al.*, 1988). Muscle and feathers mature more slowly since they are dependant upon the supply organs for their growth (Katanbaf *et al.*, 1988; Nitsan *et al.*, 1991). The functional needs of an animal change throughout its development from an embryo to adulthood (Lawrence and Fowler, 1997). The development of the pectoral muscles is related to flight; modern broilers develop flight capability by 2-3 wk of age (Provine *et al.*, 1984). Since flight capability is not required immediately after hatch, growth of the *Pectoralis* muscles occurs at a slower rate than other muscles (Hohtola and Visser, 1998). Kwakkel *et al* (1993) characterized the growth of leghorn pullets and observed that early carcass protein, fat and ash growth (before 11 wk) consisted of muscle growth, intramuscular fat deposition and skeletal growth, respectively.

The fat and protein content of broiler carcasses has been reported to increase with age (Edwards *et al.*, 1973; Evans *et al.*, 1976). Perrreault and Leeson (1992) observed an increase in carcass fat from 21 to 70 d of age but also observed a decrease in protein content over the same ages. Fat and protein content of breast muscle has been shown to increase with age (Grey *et al.*, 1983).

1.8.6 Sex effect on composition

All animals must achieve a threshold size before commencing reproduction (Liu *et al.*, 1995; Lawrence and Fowler, 1997). Energy reserves stored in the abdominal fat pad have been linked to the onset and maintenance of egg production (Bornstein *et al.*,

1984); the energy required for egg production likely accounts for the differences in fat composition between male and female broilers.

The effect of sex on the proportion of fat in broiler and turkey carcasses has been well established - females tend to have more fat than males (Carlson *et al.*, 1962; Summers *et al.*, 1965; Edwards *et al.*, 1973; Bartov *et al.*, 1974; Jackson *et al.*, 1982; Havenstein *et al.*, 1994; Gous *et al.*, 1999; Havenstein *et al.*, 2003; Latshaw and Moritz, 2009). The higher percentage of fat in females tends to increase with age (Havenstein *et al.*, 1994), likely due to increased fat requirements for supporting egg production in sexually mature females. Edwards *et al* (1973) observed that fat deposition increased rapidly after 4 wk in females while the rapid accumulation of fat did not begin in males until after 6 wk. Carcass and breast protein content has been reported to be higher in males (Summers *et al.*, 1965; Evans *et al.*, 1976; Jackson *et al.*, 1982).

1.8.7 Morphology

Prior to 1962, 83% of broilers were marketed as whole carcasses (National Chicken Council, 2008). A carcass with a full and rounded breast shape was more considered more marketable; during the 1940s and 50s several researchers investigated strain-related differences in conformation and heritability of morphology traits (Poley *et al.*, 1940; Asmundson, 1944; Bird, 1948; Abplanalp and Kosin, 1952). The shift towards cut-up and further processed marketing of broilers (41 and 48% in 2008, respectively) has highlighted the importance of *P. major* muscle morphology (National Chicken Council, 2008). As further-processed products became more popular (and profitable), fast-food restaurants and other food service institutions began to develop specific templates with breast size and shape requirements along with thickness and weight tolerances that command premium prices (Boyle, 2006). Previous studies have indicated that differences in *P. major* weight between strains are due to increased thickness of the muscle (Lubritz, 1997; Joseph *et al.*, 2002; Mehaffey *et al.*, 2006). Bartov and Plavnik (1998) indicated that providing excess dietary protein resulted in increased *P. major* muscle yield; however, no research investigating the effect of protein and energy on morphology has been reported.

1.9 INDUSTRY ISSUES

Canadian broiler chicken production is supply-managed. The allocation of production quotas, based on principles of comparative advantage, remains a contentious issue until today (Schmitz, 2008). Producers maintain control of the national governing

body, the Chicken Farmers of Canada which has authority to control imports, pricing and supply of chicken to the market (Chicken Farmers of Canada, 2008). After a period of chronic overproduction and low prices during the 1960a, the provincial chicken marketing boards formed a national association in the late 1970s (Schmitz, 2008). This national group aims to ensure that domestic demand for chicken is met by the currently 2800 Canadian broiler producers.

The chicken industry is comprised of several independently operated hatcheries, processors and feed companies (Carney and Schneider, 2005). However, decision-making in each segment of the broiler industry do not typically account for overall supply chain objectives (Zuidhof, 2004). Presently, Canadian broiler producers are paid based on live-weight of birds at processing. While this is a simple system, it is unlikely to provide clear signals to producers with regards to quality. A system in which producers are rewarded for supplying birds that meet or exceed a specified benchmark for valuable quality traits could potentially improve the efficiency and profitability of the broiler supply chain. Such a system could be based on quality traits that can be affected during the live production process and can be measured efficiently (i.e. cost effective, quick and accurate).

In consultation with a nutritionist, Alberta broiler producers choose to modify dietary energy and protein as part of the nutrition program on their farms, while still meeting the requirements for growth. Nutritional modifications that increase the amount of carcass fat represent a loss to the producer – carcass fat is expensive to produce and has little economic value (Boekholt *et al.*, 1994). Nutritional modifications that alter functional properties of meat could impact the efficiency of the supply chain.

Cut-up or further processed markets require raw products with excellent functional properties. Sex-separate rearing is utilized in some Canadian jurisdictions (Quebec) in order to take advantage of growth differences between the sexes (O'Keefe, 2006). Identifying differences in functional properties due to sex of the bird would allow processors to assign carcasses to an appropriate market destination (e.g. whole carcass, cut-up or further-processed products), which may lead to increased efficiency of the supply chain.

Finally, the period immediately prior to slaughter can affect the functional properties of meat. While this period is largely out of the producer's control, it is important to highlight the need to effectively manage broilers during this period to reduce downgrading of carcasses. Greater understanding of how each segment of the industry

contributes to final product quality would result in overall improvements to the efficiency of the broiler supply chain. Inherent differences due to the age or sex of the birds would be difficult to change, but could be exploited by supply chain participants if the cause of these differences were defined.

1.10 OBJECTIVES

1.10.1 General Objective:

At the same live weight, differences in bird conformation, composition and functional properties can make a carcass more or less valuable to processors and consumers. A live-weight based pricing scheme can lead to the production of broilers that are heavier than required by the processing industry. A value-based pricing model would provide cues that would motivate producers to raise broilers that more closely match the requirements of the Alberta processing industry. However, a value-based pricing model would require the identification of quality factors that are valuable to processors and consumers and can be influenced by producers on the farm. The objective of this thesis was to study the relationships between nutrition strategies, sex and age of broiler chickens and handling practices during the *ante mortem* period on quality and composition of chicken carcasses and breast meat. Understanding the relationships between production practices and product quality is expected to increase the efficiency of the broiler supply chain.

1.10.2 Specific Objectives:

- Define quality as it relates to poultry products
- Describe muscle structure, contraction and the conversion of muscle to meat
- Assess the effect of dietary protein and energy on total carcass composition, *P. major* meat composition and *P. major* muscle morphology
- Assess the effect of dietary protein and energy on *P. major* functional properties
- Assess the effect of holding temperature during feed withdrawal and transport on *P. major* functional properties
- Assess the effect of shackling stress on *P. major* functional properties
- Assess the effect of broiler age and sex on *P. major* composition, morphology, functional properties and total carcass composition

1.11 TABLES

Table 1.1 Summary of selected on-farm and *ante-mortem* factors that affect the quality of broiler carcasses and components.

Quality Trait	On-Farm Factors	*Ante-mortem* Factors
Ultimate pH	Diet ingredients	Holding temperature
	Lysine (Berri *et al.*, 2008)	Heat Stress (Wang *et al.*, 2009)
	Flaxseed (Betti *et al.*, 2009)	Cool Exposure (Dadgar *et al.*, 2008)
		Handling
		Struggle on shackle line (Northcutt *et al.*, 1994)
Water Holding Capacity	Diet ingredients	Holding temperature
	Lysine (Berri *et al.*, 2008)	Heat Stress (Wang *et al.*, 2009)
	Flaxseed (Betti *et al.*, 2009)	Cool Exposure (Dadgar *et al.*, 2008)
Color	Diet ingredients	Holding temperature
	Corn, wheat or milo inclusion (Smith *et al.*, 2002)	Heat Stress (Wang *et al.*, 2009)
	Lysine (Berri *et al.*, 2008)	Cool Exposure (Dadgar *et al.*, 2008)
	Flaxseed (Betti *et al.*, 2009)	Handling
		Struggle on shackle line (Ngoka and Froning, 1982; Kannan *et al.*, 1997)
Tenderness	Diet ingredients	Holding temperature
	Corn, wheat or milo inclusion (Smith *et al.*, 2002)	Heat Stress (Wang *et al.*, 2009)
	Flaxseed (Betti *et al.*, 2009)	Cool Exposure (Dadgar *et al.*, 2008)
		Handling
		Struggle on shackle line (Ngoka and Froning, 1982; Kannan *et al.*, 1997)
Composition (protein, fat and ash content)	Protein and Energy Levels (Hill and Dansky, 1954; Jackson *et al.*, 1982)	…

1.12 FIGURES

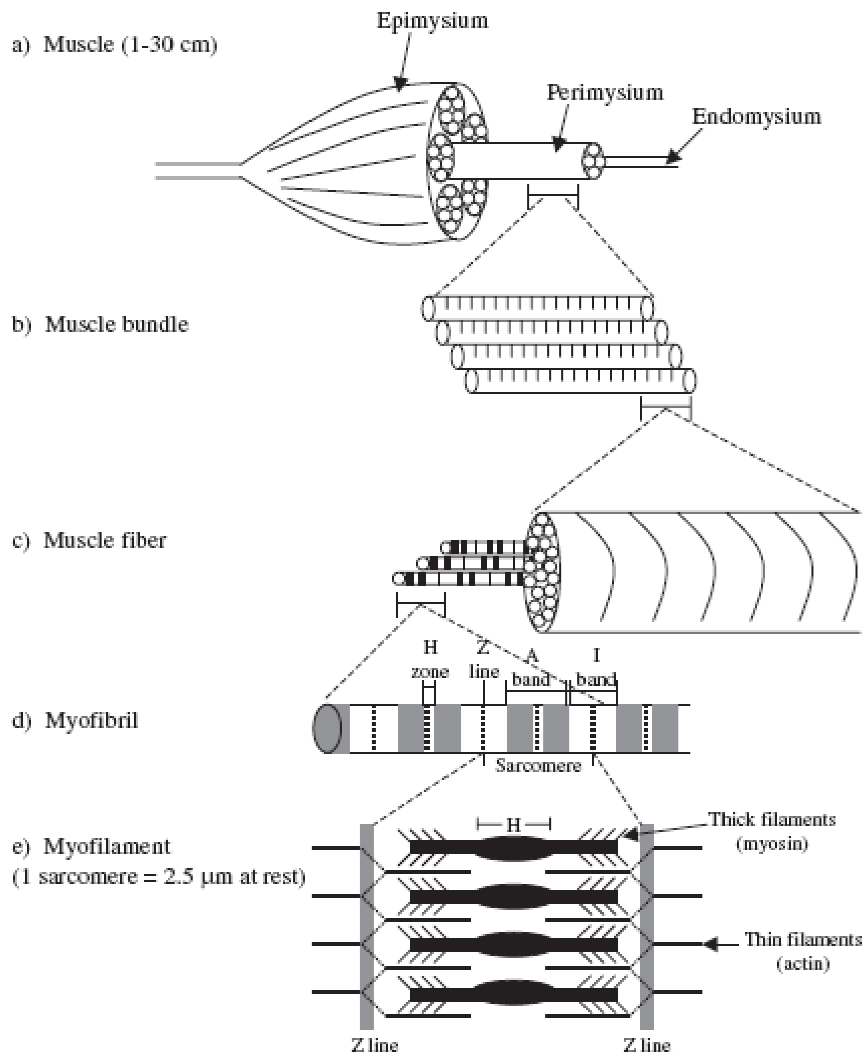

Figure 1.1. Schematic diagram of muscle structure, starting from a cross section of a whole muscle (a), including the layers of connective tissue, the muscle bundle (b), fibre (c), myofibril (d) and myofilaments (e). Reprinted from POULTRY PRODUCTS PROCESSING: AN INDUSTRY GUIDE. EBOOK by Shai Barbut. Copyright 2001 by Taylor & Francis Group LLC - Books. Reproduced with permission of Taylor & Francis Group LLC - Books in the format Dissertation via Copyright Clearance Center.

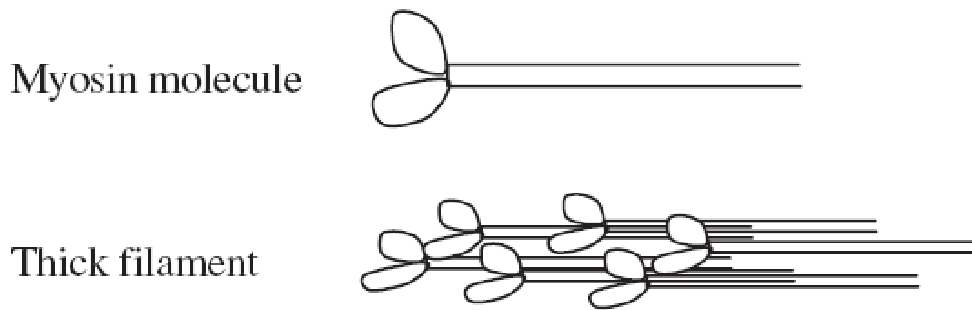

Figure 1.2. Schematic diagram of myosin molecule and thick myofilaments microstructure. Reprinted from POULTRY PRODUCTS PROCESSING: AN INDUSTRY GUIDE. EBOOK by Shai Barbut. Copyright 2001 by Taylor & Francis Group LLC - Books. Reproduced with permission of Taylor & Francis Group LLC - Books in the format Dissertation via Copyright Clearance Center.

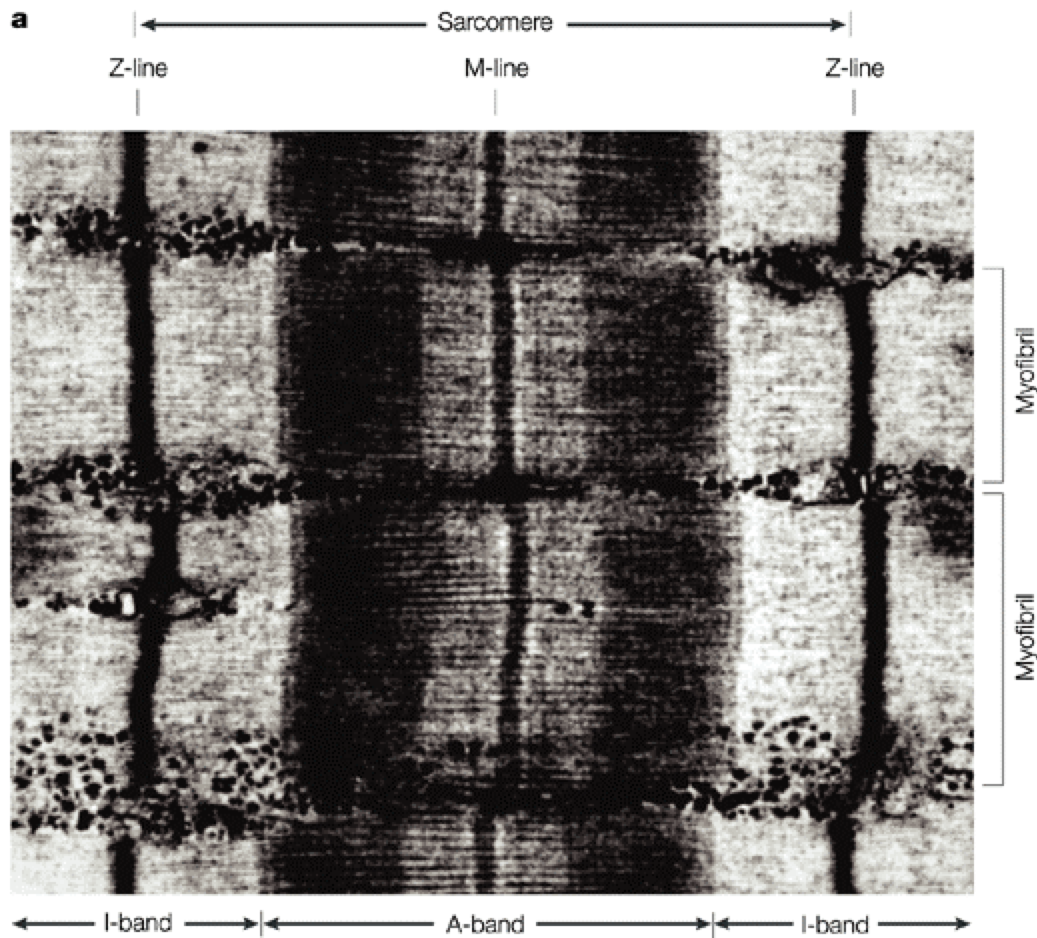

Figure 1.3. Micrograph representation of a sarcomere. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Molecular Cell Biology (4:679-689), copyright (2003)

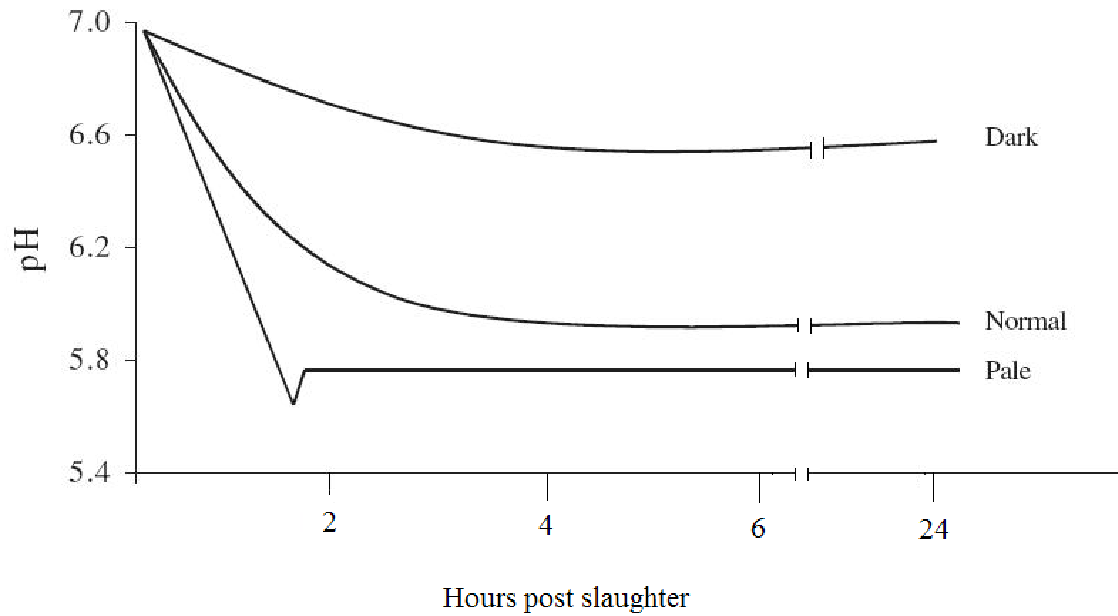

Figure 1.4. Rate and extent of Postmortem pH decline of chicken breast muscle in POULTRY PRODUCTS PROCESSING: AN INDUSTRY GUIDE. EBOOK by Shai Barbut. Copyright 2001 by Taylor & Francis Group LLC - Books. Reproduced with permission of Taylor & Francis Group LLC - Books in the format Dissertation via Copyright Clearance Center.

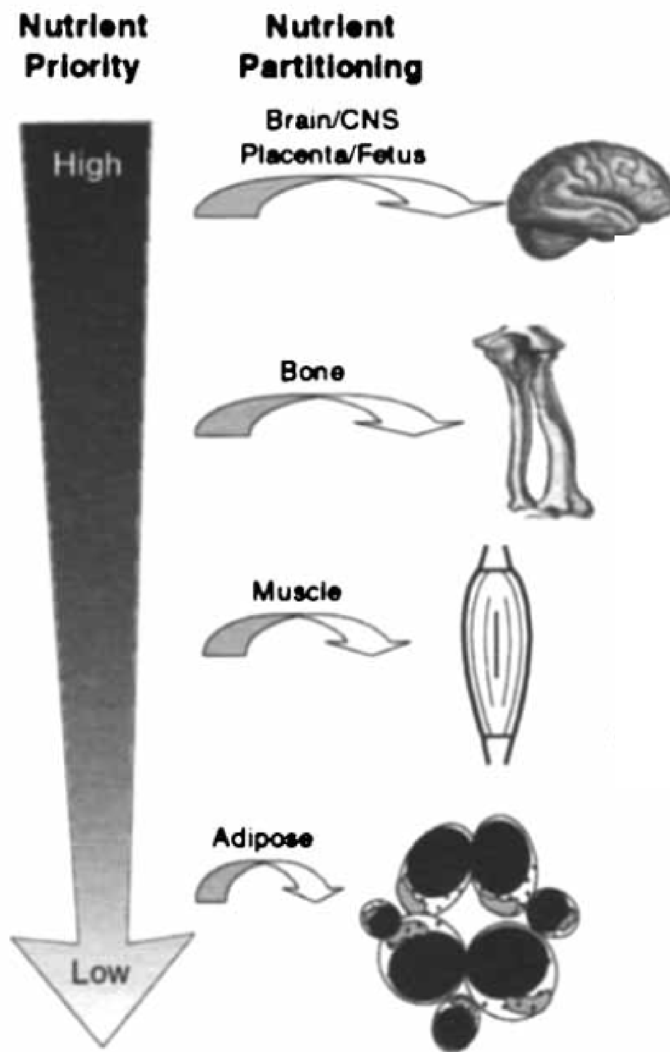

Figure 1.5. Priority of nutrient use by various tissues based upon their metabolic rate (Adapted from Hammond, 1944)). According to Hammond's nutrient partitioning model (left side of figure), those tissues with the highest metabolic rate have a greater priority for nutrient use compared to less metabolically active tissues. Reprinted in part with permission from Humphrey, B. D. and K. C. Klasing. 2004. Modulation of nutrient metabolism and homeostasis by the immune system. World's poultry science Journal 60:90-100.

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2. Effect of protein and energy level, sex and age on broiler composition and morphology

ABSTRACT

Carcass composition and *P. major* muscle composition and morphology were assessed in Cobb-Avian 48 broilers. Chicks were randomly assigned to a 2 x 2 x 3 x 3 factorial arrangement of treatments, with male and female broilers; 2 processing ages (42 and 52 d); 3 metabolizable energy (**ME**) levels which were 94, 97 and 100% of Cobb-Vantress dietary specifications for maximum growth rate and feed conversion ratio (**E94, E97** and **E100)** and 3 dietary balanced protein (**DBP**) levels of 85, 100 and 115% of the breeder specifications (**P85, P100** and **P115)** to measure carcass composition. *P. major* muscle morphology was assessed at 32, 42 and 52 d; *P. major* composition was measured at 52 d. Carcass composition was measured at 42 and 52 d. Experimental diets were provided in 3 phases; starter (11-21 d), grower (22-35 d) and finisher (36-56 d). A trade-off between percent carcass fat and protein was observed as DBP was increased from 85% to 115% of recommended levels. The percentage of carcass fat decreased as DBP increased from 85 to 115% of recommended levels (10.6 vs. 8.8 vs. 7.9%). At the same time, the percentage of carcass protein was highest in the P115 treatment (19.8%), followed by P100 (19.5%), with the lowest percentage of carcass protein observed in the P85 treatment (19.2%). Dietary ME level also resulted in a trade-off between percent carcass fat and protein. The highest percentage of carcass fat (9.5%) was observed in the E100 treatment, which also had low carcass protein (19.4%). Male broilers had a higher percentage of carcass protein and lower fat than females (19.8 vs. 19.2% and 8.3 vs. 10.0 %, respectively). *P. major* protein weight was higher in males (233 g) than females (201 g) due to increased overall *P. major* weight. Males also had a higher *P. major* fat weight (15.4 g) compared to females (11.6 g). Age and size of bird had the greatest influence on *P. major* morphology; while DBP level affected length, width and thickness of *P. major* the differences are small and likely have limited commercial significance.

Keywords: *P. major* morphology; carcass composition; dietary balanced protein; broiler; *P. major* composition; metabolizable energy

2.1 INTRODUCTION

Maximizing profit in the poultry industry is a balancing act between controlling input costs while promoting high yields of lean meat. The level of dietary protein or energy can influence the cost of a diet but also has implications for production efficiency and carcass composition.

Lean meat yield and carcass fat are influenced by the level of energy and protein and the interaction between these factors.

Growth may be described most simply as the process of getting bigger (Lawrence and Fowler, 1997). Animals change in form in response to changing physiological needs as they mature (Lawrence and Fowler, 1997). The growth rate of body components varies depending on the biological nutrient requirement of specific organ or tissue growth at a given time and is affected by nutrient supply (Kwakkel *et al.*, 1993). The effects of dietary protein and energy levels on broiler body composition have been widely reported in the literature. Fraps (1943) originally reported differences in carcass fat and protein when dietary energy and protein were manipulated. Increasing dietary energy levels has been reported to result in higher proportions of carcass fat (Hill and Dansky, 1954; Evans *et al.*, 1976; Waldroup *et al.*, 1976; Jackson *et al.*, 1982; Boekholt *et al.*, 1994; Wiseman and Lewis, 1998).

Excessive carcass fat presents a problem for the poultry industry. Fat is costly to produce and has limited economic value (Boekholt *et al.*, 1994). Manipulating diets to produce leaner broiler carcasses should practiced as long as growth of the more valuable portions of the carcass is not compromised (Kamran *et al.*, 2008). Female broilers tend to have higher carcass fat compared to males (Jackson *et al.*, 1982; Havenstein *et al.*, 1994; Gous *et al.*, 1999; Havenstein *et al.*, 2003; Latshaw and Moritz, 2009). The proportion of protein in the carcass and breast has been reported to be higher in males (Summers *et al.*, 1965; Evans *et al.*, 1976; Jackson *et al.*, 1982).

Prior to 1962, 83% of broilers were marketed as whole carcasses (National Chicken Council, 2008). The shift towards cut-up and further processed marketing of broilers (41 and 48% in 2008, respectively) has highlighted the importance of *P. major* muscle morphology (National Chicken Council, 2008). Modern food service templates have specific tolerances for *P. major* thickness, weight and shape. Previous studies have indicated that differences in *P. major* weight between strains are due to increased thickness of the muscle (Lubritz, 1997; Joseph *et al.*, 2002; Mehaffey *et al.*, 2006). Bartov and Plavnik (1998) indicated that providing excess dietary protein resulted in increased *P. major* muscle yield; however, no research investigating the effect of protein and energy on morphology has been reported.

The present experiment compared carcass and *P. major* fat, protein and ash from broilers fed graded levels of dietary protein and energy. The effects of sex and age of broilers and their interaction with protein and energy level were also measured.

2.2 MATERIALS & METHODS

2.2.1 Experimental Design

The present experiment is an extension of a larger project investigating the impact of dietary balanced protein (**DBP)** and metabolizable energy (**ME**) levels on broiler performance, yield and carcass composition, with a 2 x 2 x 3 x 5 factorial treatment arrangement (Zuidhof *et al.*, 2008). The project had 2 sexes; 2 levels of early nutrition (0 – 11 d); and after 11 d, three levels of ME which were 94, 97 and 100% of Cobb-Vantress ME recommendations for maximum growth rate and feed conversion ratio (**E94, E97, E100**, respectively). The five DBP levels were 85, 92.5, 100, 107.5 and 115% of Cobb-Vantress protein recommendations for maximum growth rate and feed conversion ratio, balanced for 6 amino acids: Methionine, Methionine + Cysteine, Tryptophan, Threonine, and Arginine (**Met, Met+Cys, Trp, Thr and Arg**). Base diet specifications (100% level) are presented in Table 2.1.

Carcass composition was measured on a subset of birds and consisted of a 2 x 2 x 2 x 3 x 3 factorial arrangement of treatments, with 2 sexes, 2 processing ages (42 and 52 d), 3 ME levels (E94, E97 and E100) and 3 DBP levels (85, 100 and 115% of Cobb-Vantress protein recommendations for maximum growth rate and feed conversion ratio (**P85**, **P100** and **P115**, respectively)). *P. major* morphology was measured at 3 processing ages (32, 42, and 52 d). *P. major* composition was measured at 52 d.

2.2.2 Experimental Diets

To minimize variation in experimental diets due to ingredient variability, four basal diets per phase were formulated (Table 2.2), and then blended (Table 2.3) to make the 9 treatment diets for the starter (12 – 21 d), grower (22-35 d) and finisher (36 to 56 d) phases. The composition of the major dietary ingredients was measured before the formulation for the four basal diets (A, B, C and D, Table 2.3) was finalized (Zuidhof *et al.*, 2008). The ingredient composition of all experimental diets is presented in Table 2.4. Crude protein and amino acids of the main feed ingredients were determined prior to finalizing diet formulations. Diets were provided as pellets.

Crude protein content (Leco Truspec CN3402, LECO Corporation., St. Joseph, MI 49085), gross energy (IKA Calorimeter C 5000, IKA., Werke Staufen, Germany), amino acids (Sedgwick *et al.*, 1991), dry matter and ash (AOAC, 2006) was measured for all diets in all phases. Apparent metabolizable energy (**AME**) was measured for each diet (Scott and Boldaji, 1997; Scott and Hall, 1998). AME values for grower diets A, C and D were 4.3, 5.6 and 6.0% lower than targets, possibly due to variation in ingredient ME levels (Zuidhof *et al.*, 2008). Ingredient ME levels were not evaluated prior to formulation (Zuidhof *et al.*, 2008). Expected and actual protein and ME contents of the experimental diets are presented in Table 2.5.

2.2.3 Stocks and Management

The Canadian Council of Animal Care (1993) guidelines for animal care were met throughout the duration of the experiment and all protocols were approved by the University of Alberta Animal Care and Use Committee – Livestock. Chicks were feather sexed and identified with neck tags (Heartland Animal Health, Fair Play, MO 65649) at hatch prior to placement. Birds were housed sex-separately throughout the experiment. During the early nutrition phase (0-11 d), 3424 Cobb x Avian 48 broiler chicks were randomly distributed among 16 floor pens. At 12 d birds were distributed to 60 floor pens. Prestarter nutrition treatments were nested within pen after the early nutrition phase. A 23L: 1D lighting schedule was provided for the first 3 d followed by 20L: 4D to 56 d. Access to feed and water (nipple drinkers) was provided *ad libitum* for the duration of the experiment.

2.2.4 Processing and Sample Preparation

Eight birds per DBP*ME*Sex interaction were processed at 42 and 52 d of age (n=144 per age). Birds were crated for a 12 hr feed withdrawal period within the rearing facility and were transported to the processing facility immediately prior to slaughter. The birds were electrically stunned, slaughtered by a ventral neck cut and bled for 2 minutes. After scalding (63°C) for 45 seconds, carcasses were mechanically de-feathered. Carcasses were manually eviscerated and held at 4°C for 24 h prior to deboning. Sex was confirmed during evisceration. Morphology measurements were taken immediately following deboning. Eviscerated carcasses (without giblets) were frozen at -20°C for 2 to 8 weeks prior to analysis of composition. Fat pads were completely removed from the carcass. At 52 d, a subsample of *P. major* (~30 g) was removed from the carcass at deboning for analysis of composition. *P. major* samples were frozen at -20°C for 12 wk prior to analysis.

2.2.5 Carcass and P. major Composition

Carcass composition samples were prepared for analysis using the method described by Renema *et al.* (1999) and Yu *et al.* (1990). *P. major* samples were freeze-dried for 4 d and corrections were made for moisture loss during freeze-drying. Dried *P. major* samples were homogenized in an industrial blender. Crude protein for carcass and *P. major* samples was determined in duplicate using a Leco Truspec analyzer. Dry matter (carcass and *P. major*) and ash (carcass) were determined in duplicate using Association of Official Analytical Chemists official methods 930.15 and 942.05, respectively (AOAC, 2006). The total lipid content of *P. major* and carcass samples was determined gravimetrically. Organic solvents (chloroform and hexane) were used to separate the lipid fraction from the freeze-dried *P. major* and carcass samples.

2.2.6 Morphology Measurements

Total length of the fillet is defined along a diagonal axis running from the highest point of the cranial end of the left fillet to the tip of the keel at the caudal end of the fillet (Figure 2.1). The cranial thickness is defined as the thickness of the *P. major* fillet at the point 15% below the cranial edge of the fillet along the diagonal axis (Figure 2.1). Middle thickness is defined as the thickness at the point located halfway along the length of the diagonal axis (Figure 2.1). Finally, caudal thickness is defined as the thickness of the *P. major* fillet at the point 15% above the caudal end (keel) of the *P. major* fillet along the diagonal axis (Figure 2.1). Width of the *P. major* fillet was measured along a horizontal axis at the same cranial, middle and caudal points used for thickness measurements (Figure 2.2). Width was measured mediolaterally, from the keel (or in the case of the cranial point, from the edge of the fillet closest to the keel) to the outer edge of the fillet. Cranial width was measured along a horizontal axis intersecting point a, 15% below the cranial edge of the fillet. Middle width was measured along the axis intersecting point b, or the midpoint of the fillet. Caudal width was measured along an axis intersecting the point c, 15% above the caudal edge of the fillet. Thickness measurements from 42 d were not reported due to measurement error.

2.2.7 Statistical Analysis

Carcass composition and morphology data was analyzed as a 4-way analysis of covariance using the mixed model procedure of SAS® (SAS Institute Inc., 2008) using the following statistical model:

$$Y_{ijklm}=\mu + P_i + E_j + PE_{ij} + S_k + SP_{ik} + SE_{jk} + A_l + AP_{il} + AE_{jl} + AS_{kl} + \beta(BW_{ijklm} - BW_a) + \varepsilon_{ijklm}$$

Where Y_{ijklm}= variable measured for the mth bird, μ = overall mean, P_i = effect of the ith DBP level; E = effect of the jth ME level; PE_{ij} =interaction between DBP and ME; S_k = effect of the kth sex; SP_{ik} = interaction between sex and DBP; SE_{jk} = interaction between sex and ME; A_l = effect of lth age; AP_{il} = interaction between age and DBP; AE_{jl} = interaction between age and ME; AS_{kl} = interaction between sex and age; $\beta(BW_{ijklm} - BW_a)$ = a covariate coefficient which is multiplied by the difference between individual body weight (BW_{ijklm}) and average BW (BW_a) to account for the BW effect; and ε_{ijklm} = residual error component.

P. major composition data was analyzed as a 3-way analysis of variance using the following statistical model:

$Y_{ijkl}=\mu + P_i + E_j + PE_{ij} + S_k + SP_{ik} + SE_{jk} + \varepsilon_{ijkl}$ where Y_{ijkl}= variable measured for the mth bird, μ = overall mean, P_i = effect of the ith DBP level; E = effect of the jth ME level; PE_{ij} =interaction between DBP and ME; S_k = effect of the kth sex; SP_{ik} = interaction between sex and DBP; SE_{jk} = interaction between sex and ME; and ε_{ijkl} = residual error component.

All composition data was analyzed with bird as a random effect to account for within bird variation. Morphology data was analyzed with the person making the measurements as a random effect. Least squares means were evaluated with pairwise comparisons, and were reported as significantly different at the P ≤0.05 level. SEM for the group with the least number of birds was reported.

2.3 RESULTS AND DISCUSSION

2.3.1 Carcass Composition

2.3.1.1 *Carcass Fat*

The percentage of carcass fat decreased as protein level increased from P85 to P100 to P115 (10.6 vs. 8.8 vs. 7.9% respectively, Table 2.6). This is consistent with previous studies that reported increasing dietary protein resulted in lower carcass fat content (Fraps, 1943; Bartov *et al.*, 1974; Jackson *et al.*, 1982; Smith *et al.*, 1998; Bregendahl *et al.*, 2002). Energy and protein level affected both the absolute weight and percentage of carcass fat. The percentage of carcass fat was highest in the E100 treatment (9.53%), with no difference between E94 and E97 (8.95 and 8.92%, respectively, Table 2.7). Several previous studies have also reported that increasing dietary ME increases carcass fat deposition (Jackson *et al.*, 1982; Campbell *et al.*, 1988; Boekholt *et al.*, 1994; Wiseman and Lewis, 1998). The absolute weight of carcass fat also decreased as protein level increased from P85 to P100 to P115 (205.2 vs. 167.4 vs. 151.0 g respectively, Table 2.6). However, an interaction between DBP and ME level was observed for absolute weight of carcass fat. The combination of P85*E100 resulted in the greatest fat deposition (222.1 g, Table 2.8). As the protein level of a diet is decreased relative to energy, birds will increase their overall feed intake to meet their amino acid requirement; excess energy is deposited as fat (Bartov, 1979). Providing protein above recommended levels (P115) decreased fat deposition even as ME level increased between 94, 97 and 100% of recommended levels (151.6, 150.7 and 150.7 g, respectively; Table 2.8). Carcass fat was also low in the P100*E94 treatment (154.6 g, Table 2.8). The decrease in carcass fat associated with high protein-low energy diets in the present experiment could be related to the energetic cost associated with deamination of excess amino acids (Bartov, 1979). Bartov (1985) theorized that decreases in carcass fat when feeding diets high in protein were related to the energy expenditure associated with eliminating excess nitrogen.

Higher percentages of carcass protein and lower carcass fat was observed in males compared to females (19.81 vs. 19.17% and 8.29 vs. 9.97 %, respectively, $p<.0001$; Table 2.9). This observation has been reported by several authors (Jackson *et al.*, 1982; Havenstein *et al.*, 1994; Gous *et al.*, 1999; Havenstein *et al.*, 2003; Latshaw and Moritz, 2009). Higher fat

deposition in females is associated with energy reserves required for reproduction (Bornstein *et al.*, 1984). Although broilers are processed well before sexual maturity, the increase in fat deposition in females could be offset by slaughtering female broilers at earlier ages. This would require sex-separate rearing but may improve the efficiency of the supply chain.

2.3.1.2 *Carcass Protein*

As DBP level increased stepwise from P85 to P100 to P115 the percentage of carcass protein also increased (19.2 vs. 19.5 vs. 19.8 %, respectively; Table 2.6). This agrees with results reported by Jackson *et al.* (1982). On an absolute basis, P85 limited protein deposition (364.0 g; Table 2.6) while no difference was observed between P100 and P115 (371.5 and 377.1 g, respectively). This result agrees with Lopez *et al.* (2007) who reported that carcass protein is limited by genetic potential when amino acids are supplied in an appropriate amount and balance. The lack of additional carcass protein deposition in the P115 treatment in the current experiment supports the conclusion that protein accretion is limited by genetics.

The percentage of carcass protein was highest in the E94 (19..7%) treatment with no difference observed between the E97 and E100 treatments (19.4 and 19.4%, respectively; Table 2.7). In contrast, Leeson and Summers (2005) observed that nutrition had minimal effects on the absolute values of protein or meat in a carcass; leaner carcasses are not the result of greater protein deposition but are the product of decreased fat deposition. Carcass protein increased from 18.57% to 20.41% between 42 and 52 d of age ($P<0.0001$, Table 2.10). No effects on carcass ash or fat were observed. These results indicate that the major component of growth between these ages was muscle deposition. This is not surprising, as Zuidhof *et al.* (2005) reported that breast muscle yield increases at a greater rate relative to the carcass as a whole as broilers increase in size. Further, Kwakkel *et al*. (1993) described growth in component fractions (protein, fat and ash) of leghorn pullets as diphasic, with the first 11 wk associated with muscle growth, intramuscular fat and skeletal growth. After 11 wk growth was associated with the reproductive tract and abdominal fat pad (Kwakkel *et al.*, 1993). Although growth rates are much higher in modern broiler strains compared to leghorn strains, growth in component fractions is presumably similar.

2.3.1.3 *Carcass Ash*

Percent carcass ash was not affected by ME level. No differences were observed in percent carcass ash content at P85 and P100 levels (2.36 and 2.40 %, respectively; Table 2.6), however, P115 resulted in higher carcass ash (2.52%). Absolute weight of carcass ash followed a similar pattern, with no difference between the P85 and P100 treatments (44.72 and 45.61 g, respectively) with higher carcass ash in the P115 treatment (48.11 g, Table 2.6). Carcass ash is

associated with skeletal development which is related functionally to the amount of fat and protein in a carcass (Kwakkel *et al.*, 1993). Higher carcass ash was also observed in males relative to females (2.53 vs. 2.32%, Table 2.9). Heavier BW and muscle weights are generally observed in males compared to females (Lonergan *et al.*, 2003). A larger skeletal system is required to support increased BW which accounts for the increased ash in males; increased muscle mass will also contribute to higher percentages of carcass protein.

2.3.2 *P. major* Composition

Dietary ME level affected the percentage of protein in *P. major*. Percent protein was higher in the E94 (39.23%) compared to the E100 (37.46%) treatment while E97 (38.33%) was not different from either (Table 2.11). The percentage of fat in *P. major* tended to increase as ME level increased but was not significant (P=0.9576). The tendency for percentage fat to increase as ME increased is likely responsible for the decrease in percent protein. On an absolute basis, ME did not affect the weights of either fat or protein in *P. major*. Protein accretion is limited by genetics and requires sufficient and balanced amino acid supplies through diet (Lopez *et al.*, 2007).

Sex of bird did not affect the percentage of fat or protein in *P. major* (Table 2.11). However, males had more *P. major* protein (233.2 g) and fat (15.4 g) on an absolute weight basis than females (201.2 g and 11.6 g) due to greater *P. major* weight at processing (427.4 g males, 375.5 g females). No effects of DBP level were observed for *P. major* composition traits (Table 2.11).

2.3.3 Morphology

Dietary protein affected length, width and thickness of *P. major*. The P115 treatment resulted in greater total length compared to the P85 treatment while P100 total length was not different from either (173.50 vs. 170.93 and 172.7 mm, respectively; Table 2.12). Width at the midpoint of the breast was also limited by P85 (77.84 mm) with no difference between P100 and P115 (79.85 and 80.34 mm, respectively). Finally, thickness at the cranial end of *P. major* was limited by P85 (25.47) with no difference between P100 and P115 (26.28 and 26.86 mm, respectively). Robinson *et al* (2003) reported that low crude protein diets (19%) reduced carcass frame size (as measured by shank length). The P85 treatment in the current experiment had 20% crude protein during the starter phase and less than 17% during the finisher phase (Table 2.5); P85 appears to have limited frame size, as evidenced by reduced length and width of *P. major* and is further supported by the decrease in absolute weight and percent of carcass ash (reduced skeletal development) in the same treatment.

Although DBP levels affected *P. major* dimensions, bird size and age also influenced total length. As age increased from 32 to 52 d, total length of the breast also increased (165.26 vs. 170.75 vs. 180.90 mm, respectively; Table 2.12). Furthermore, as body weight increased by 100 g, total length of the breast increased by 1.57 mm. Width at the midpoint of the breast increased by 1.46 mm as body weight increased by 100 g (Table 2.12).

Strain has been shown to influence *P. major* dimensions (Lubritz, 1997; Joseph *et al.*, 2002; Mehaffey *et al.*, 2006). The differences in total length attributed to nutritional treatments, while statistically significant, are practically quite small and would likely not have commercial significance. A more practical approach might be to select a strain with *P. major* morphology that fits fast food templates.

2.4 CONCLUSIONS

Dietary composition dramatically influenced the leanness of broiler carcasses. Altering protein or energy levels can result in excess fat deposition or reduced frame size which may negate cost savings by reducing the value of the end-product. Differences in male and female growth could be exploited by raising male and female broilers separately; slaughtering female broilers at earlier ages may limit decreases in carcass value due to excess fat deposition. A thorough cost-benefit analysis would be required to assess the effect of sex-separate rearing on the efficiency of the broiler supply chain. Composition of *P. major* fillets may be difficult to change by altering dietary protein and energy. *P. major* morphology differences due to nutritional treatments were limited; age, bird size and strain are likely to have a greater influence on breast muscle morphology.

2.5 TABLES

Table 2.1. Nutrient composition of the 100%[1] Energy, 100%[1] DBP diets for prestarter (0-10 d), starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

	Prestarter				
Calculated nutrient composition	HighPS[2]	LowPS[2]	Starter	Grower	Finisher
ME (kcal/kg)	3,150	2,976	3,150	3,200	3,250
Protein (%)	22.5	21	22.50	20.00	19.00
Arginine-total (%)	1.43	1.26	1.40	1.30	1.20
Lysine-total (%)	1.35	1.20	1.40	1.20	1.10
Methionine-total (%)	0.55	0.49	0.60	0.60	0.50
M+C-total (%)	0.99	0.89	1.00	0.90	0.90
Tryptophan-total (%)	0.22	0.19	0.30	0.30	0.30
Threonine-total (%)	0.89	0.79	0.90	0.80	0.80
Calcium (%)	0.90	0.90	0.90	0.90	0.80
Phosphorous Available (%)	0.45	0.45	0.50	0.40	0.40
Sodium (%)	0.20	0.20	0.20	0.20	0.20
Chloride (%)	0.20	0.20	0.50	0.40	0.40
Potassium (%)	0.65	0.65	1.00	0.90	0.80

Table modified from Zuidhof, M. J., F. I. L. Hernandez, D. R. Korver, and R. A. Renema. 2008. An integrated nonlinear analysis of nutritional effects on broiler performance. Pages 29-63 *in* Final Report to Funders: Value-based marketing decision support through characterization of growth, fatness and yield. Alberta Agriculture and Rural Development, Edmonton, AB.

[1] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2]HighPS: - prestarter high; LowPS – prestarter low.

Table 2.2. Nutritional composition of formulated diets used to blend 9 treatment diets for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases for 85, 100 and 115%[1] dietary balanced protein (DBP) and 94, 97 and 100%[1] Metabolizable Energy (ME) combinations.

	Starter				Grower				Finisher			
Item	A[2]	B[3]	C[4]	D[5]	A[2]	B[3]	C[4]	D[5]	A[2]	B[3]	C[4]	D[5]
Nutrient composition	Calculated											
ME (kcal/kg)	2,961	3,150	2,961	3,150	3,008	3,200	3,008	3,200	3,055	3,250	3,055	3,250
Protein (%)	19.13	19.13	25.88	25.88	17.00	17.00	23.00	23.00	16.15	16.15	21.85	21.85
Fat (%)	3.04	5.42	1.98	4.30	3.93	6.97	3.11	6.18	4.69	7.00	3.86	6.50
Fiber (%)	5.25	4.51	5.22	4.40	5.15	5.06	5.24	5.18	5.13	4.22	5.19	4.61
Arginine-total (%)	1.22	1.22	1.64	1.64	1.10	1.10	1.48	1.48	0.99	0.99	1.35	1.35
Lysine (%)	1.15	1.15	1.55	1.55	1.02	1.02	1.38	1.38	0.94	0.94	1.27	1.27
Methionine-total (%)	0.49	0.49	0.68	0.68	0.47	0.47	0.66	0.66	0.45	0.45	0.63	0.63
M+C-total (%)	0.84	0.84	1.14	1.14	0.80	0.80	1.08	1.08	0.77	0.77	1.04	1.04
Threonine-total (%)	0.76	0.76	1.02	1.02	0.70	0.70	0.94	0.94	0.64	0.64	0.86	0.86
Tryptophan-total (%)	0.27	0.26	0.38	0.37	0.24	0.24	0.33	0.33	0.23	0.22	0.32	0.31
Calcium (%)	0.90	0.90	0.90	0.90	0.88	0.88	0.88	0.88	0.84	0.84	0.84	0.84
Phosphorous Avail (%)	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.40	0.40	0.40	0.40
Sodium (%)	0.23	0.23	0.23	0.23	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
Chloride (%)	0.47	0.47	0.49	0.48	0.43	0.43	0.44	0.44	0.41	0.41	0.43	0.42
Potassium (%)	0.98	0.90	1.12	1.06	0.88	0.85	1.02	1.00	0.86	0.77	0.98	0.93
Choline (mg/kg)	1490	1425	1533	1522	1373	1297	1427	1356	1356	1297	1370	1343
Vitamin E (IU/kg)	64	63	60	59	65	63	61	60	65	64	61	61
Nutrient composition	Analyzed											
ME (kcal/kg)	3,009	3,169	3,014	3,094	2,909	3,119	2,842	3,008	3,022	3,137	2,972	3,171
Protein (%)	20.70	19.80	27.80	27.80	16.39	17.41	23.41	22.31	16.09	16.78	20.76	21.52
Arginine-total (%)	-	-	-	-	1.12	1.10	1.24	1.34	1.06	1.02	1.34	1.36
Lysine-total (%)	-	-	-	-	1.15	1.13	1.12	1.09	0.90	1.26	1.34	1.28
Methionine-total (%)	-	-	-	-	0.54	0.42	0.66	0.63	0.45	0.44	0.66	0.64
Threonine-total (%)	-	-	-	-	0.72	0.71	0.77	0.77	0.62	0.67	0.81	0.72

Modified from Zuidhof, M. J., F. I. L. Hernandez, D. R. Korver, and R. A. Renema. 2008. An integrated nonlinear analysis of nutritional effects on broiler performance. Pages 29-63 *in* Final Report to Funders: Value-based marketing decision support through characterization of growth, fatness and yield. Alberta Agriculture and Rural Development, Edmonton, AB.

[1]Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2]Diet A: 85% of base DBP; 94% base ME.
[3]Diet B: 85% of base DBP; 100% base ME.
[4]Diet C: 115% of base DBP; 94% base ME.
[5]Diet D: 115% of base DBP; 100% base ME.

Table 2.3. Blending[1] specifications for 9 experimental diets with combinations of 94, 97 or 100%[2] metabolizable energy (ME) and 85, 100 or 115%[2] dietary balanced protein (DBP) for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

	DBP Level (%)		
ME Level (%)	85	100	115
94	A[3]	AC	C[5]
97	AB	ABCD	CD
100	B[4]	BD	D[6]

Modified from Zuidhof, M. J., F. I. L. Hernandez, D. R. Korver, and R. A. Renema. 2008. An integrated nonlinear analysis of nutritional effects on broiler performance. Pages 29-63 *in* Final Report to Funders: Value-based marketing descion support through characterization of growth, fatness and yield. Alberta Agriculture and Rural Development, Edmonton, AB.

[1] For each phase, diets A, B, C, and D were formulated. Each instance of A, B, C or D indicates than an equal amount of that diet was present in the experimental diet.
[2] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3]Diet A: 85% of base DBP; 94% base ME.
[4]Diet B: 85% of base DBP; 100% base ME.
[5]Diet C: 115% of base DBP; 94% base ME.
[6]Diet D: 115% of base DBP; 100% base ME.

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Table 2.4. Ingredient composition of experimental diets containing combinations of 94, 97 or 100%[1] metabolizable energy (ME) and 85, 100 or 115%[1] dietary balanced protein (DBP) for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

ME (%)	Phase	DBP (%)	Corn	Wheat	Soybean meal	Wheat bran	Canola oil	DL-Met	L-Lys HCL	L-Thr	Arg	Dicalcium phosphate	Calcium carbonate	Sodium chloride	Cocci-diostat	Anti-biotic	Pre-mix[2]
			%														
94	Starter	85	47.0	0.0	22.0	25.0	0.35	0.16	0.25	0.01	0.02	1.26	1.36	0.56	0.05	0.05	0.50
		100	23.5	19.1	26.8	24.5	0.32	0.23	0.29	0.03	0.01	1.19	1.38	0.56	0.05	0.05	0.50
		115	0.0	38.3	31.6	24.1	0.28	0.29	0.33	0.05	0.00	1.13	1.41	0.56	0.05	0.05	0.50
	Grower	85	50.0	2.0	16.2	25.0	1.14	0.18	0.28	0.05	0.08	1.13	1.41	0.48	0.05	0.05	0.50
		100	30.0	17.5	20.7	25.0	1.16	0.25	0.32	0.07	0.07	1.07	1.43	0.49	0.05	0.05	0.50
		115	10.0	32.9	25.1	25.0	1.17	0.31	0.35	0.10	0.06	1.01	1.45	0.49	0.05	0.05	0.50
	Finisher	85	52.8	0.0	15.1	25.0	1.84	0.16	0.22	0.01	0.02	1.04	1.36	0.46	0.05	0.05	0.50
		100	31.3	17.7	18.8	25.0	1.87	0.23	0.26	0.04	0.01	0.98	1.39	0.46	0.05	0.05	0.50
		115	9.8	35.3	22.6	25.0	1.90	0.29	0.29	0.06	0.00	0.92	1.42	0.46	0.05	0.05	0.50
97	Starter	85	43.5	5.0	21.8	22.4	1.67	0.16	0.26	0.01	0.03	1.31	1.34	0.56	0.05	0.05	0.50
		100	21.8	22.3	26.9	21.7	1.60	0.23	0.29	0.03	0.01	1.25	1.36	0.56	0.05	0.05	0.50
		115	0.0	39.6	32.1	21.0	1.53	0.29	0.33	0.05	0.00	1.19	1.38	0.56	0.05	0.05	0.50
	Grower	85	44.7	6.0	15.8	25.0	2.79	0.18	0.30	0.06	0.08	1.14	1.42	0.48	0.05	0.05	0.50
		100	24.9	21.2	20.3	25.1	2.80	0.25	0.33	0.08	0.08	1.07	1.44	0.49	0.05	0.05	0.50
		115	5.0	36.5	24.8	25.1	2.82	0.32	0.36	0.10	0.07	1.01	1.46	0.49	0.05	0.05	0.50
	Finisher	85	49.9	5.0	14.9	21.6	3.13	0.16	0.23	0.02	0.03	1.10	1.33	0.46	0.05	0.05	0.50
		100	29.2	21.1	18.9	22.3	3.22	0.23	0.26	0.04	0.02	1.03	1.36	0.46	0.05	0.05	0.50
		115	8.4	37.1	22.9	22.9	3.32	0.29	0.29	0.06	0.00	0.96	1.40	0.47	0.05	0.05	0.50
100	Starter	85	40.0	10.0	21.5	19.7	3.00	0.17	0.27	0.02	0.04	1.35	1.33	0.56	0.05	0.05	0.50
		100	20.0	25.5	27.1	18.8	2.89	0.23	0.29	0.04	0.02	1.30	1.34	0.56	0.05	0.05	0.50
		115	0.0	41.0	32.6	17.8	2.77	0.30	0.32	0.05	0.00	1.25	1.35	0.56	0.05	0.05	0.50
	Grower	85	39.4	10.0	15.4	25.0	4.43	0.19	0.31	0.06	0.09	1.14	1.42	0.49	0.05	0.05	0.50
		100	19.7	25.0	20.0	25.1	4.45	0.25	0.34	0.09	0.08	1.08	1.45	0.49	0.05	0.05	0.50
		115	0.0	40.0	24.5	25.2	4.47	0.32	0.37	0.11	0.07	1.01	1.47	0.49	0.05	0.05	0.50
	Finisher	85	47.1	10.0	14.8	18.3	4.43	0.16	0.24	0.03	0.04	1.16	1.31	0.46	0.05	0.05	0.50
		100	27.0	24.5	19.0	19.5	4.58	0.23	0.26	0.04	0.02	1.08	1.34	0.46	0.05	0.05	0.50
		115	6.9	38.9	23.2	20.8	4.73	0.30	0.29	0.06	0.00	1.00	1.38	0.47	0.05	0.05	0.50

[1] Base specifications (100% ME and 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.

[2] Premix provided the following (per kg of diet): vitamin E, 3 g; lignosulfonate binder, 6 g; choline chloride, 5 g (provided 400 mg) and broiler premix, 5 g containing: vitamin A (as retinyl acetate), 10,000 IU; vitamin D_3 (as cholecalciferol), 2,500 IU; vitamin K (menadione sodium bisulfite), 2.0 mg; vitamin E (as D-α-tocopherol), 35 IU; biotin, 0.18 mg; folic acid , 0.8 mg; niacin, 65 mg; D-pantothenic acid, 14 mg; pyridoxine, 4 mg ; riboflavin, 5 mg; thiamine, 2 mg; vitamin B_{12}, 20 µg; iron, 100 mg, Mn, 70 mg; Cu 8.5 mg; Se, 0.10 mg; zn, 80 mg; I, 0.5 mg, 5

Table 2.5. Expected and actual protein and energy composition of broiler starter (11-21 d), grower (22-35 d) and finisher (36-56 d) experimental diets varying in dietary balanced protein and metabolizable energy.

	Dietary Balanced Protein (% of recommended) [1]																	
	85						100						115					
	Starter		Grower		Finisher		Starter		Grower		Finisher		Starter		Grower		Finisher	
ME Level[1]	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual
	Dietary Energy Level (kcal/kg)																	
94	2964	3009	3011	2909	3058	3022	2964	3012	3011	2876	3058	2997	2964	3014	3011	2842	3058	2972
97	3056	3089	3104	3014	3153	3080	3056	3072	3104	2970	3153	3076	3056	3054	3104	2925	3153	3072
100	3150	3169	3200	3119	3250	3137	3150	3132	3200	3064	3250	3154	3150	3094	3200	3008	3250	3171
	Protein Level (%)																	
94	19.1	20.7	17.0	16.4	16.2	16.1	22.5	24.3	20.0	19.9	19.0	18.4	25.9	27.8	23.0	23.4	21.9	20.8
97	19.1	20.3	17.0	16.9	16.2	16.4	22.5	24.0	20.0	19.9	19.0	18.8	25.9	27.8	23.0	22.9	21.9	21.1
100	19.1	19.8	17.0	17.4	16.2	16.8	22.5	23.8	20.0	19.9	19.0	19.2	25.9	27.8	23.0	22.3	21.9	21.5

[1] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio

Table 2.6. Covariate analysis[1] of carcass ash, fat and protein (% of eviscerated body weight and absolute weight) of Cobb x Avian 48 broilers in response to dietary balanced protein level.

Composition Traits	Dietary Balanced Protein[2] 85	100	115	Covariate Eviscerated Body Weight[3]	SEM	Pr > F
	--------%[4]--------			%/g		
Ash	2.36^{b}	2.40^{b}	2.52^{a}	0.0002	0.04	0.005
Fat	10.6^{a}	8.8^{b}	7.9^{c}	0.0018	0.17	<0.0001
Protein	19.2^{c}	19.5^{b}	19.8^{a}	-0.0002	0.10	<0.0001
	--------g--------			g/g		
Ash	44.7^{b}	45.6^{b}	48.1^{a}	0.03	0.69	0.001
Fat	205.2^{a}	167.4^{b}	151.0^{c}	0.13	3.65	<0.0001
Protein	364.0^{b}	371.5^{a}	377.1^{a}	0.19	2.26	0.0002

[a-c] Means within a row with different superscripts differ significantly ($P < 0.05$)
[1] Means adjusted for body weight at evisceration
[2] Base specifications (100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3] Slope coefficients from covariate analysis
[4] Percent of eviscerated body weight

Table 2.7. Covariate analysis[1] of carcass ash, fat and protein (% of eviscerated body weight and absolute weight) of Cobb x Avian 48 broilers in response to metabolizable energy level.

	ME[1] (% of recommended)					
Composition Traits	94	97	100	Eviscerated Body Weight[3]	SEM	Pr > F
	----------------------------%[4]----------------------------			%/g		
Ash	2.43	2.46	2.39	0.0002	0.04	0.329
Fat	9.0[b]	8.9[b]	9.5[a]	0.0018	0.16	0.012
Protein	19.7[a]	19.4[b]	19.4[b]	-0.0002	0.10	0.039
	----------------------------g----------------------------			g/g		
Ash	46.1	47.0	45.4	0.03	0.65	0.218
Fat	169.3[b]	169.8[b]	184.5[a]	0.13	3.48	0.002
Protein	374.7[a]	370.1[ab]	367.6[b]	0.19	2.13	0.049

[a-c] Means within a row with different superscripts differ significantly ($P < 0.05$)
[1] Means adjusted for body weight at evisceration
[2] Base specifications (100% ME) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3] Slope coefficients from covariate analysis
[4] Percent of eviscerated body weight

Table 2.8 Interaction between various combinations of dietary balanced protein (DBP) and Metabolizable Energy (ME) on carcass ash, fat and protein (% of eviscerated body weight and absolute weight) of Cobb x Avian 48 broilers

		DBP[1] (% of recommended)				
Composition Trait	ME[1]	85	100	115	SEM	Pr > F
Ash (%)[2]	94	2.39	2.39	2.51	0.06	0.303
	97	2.42	2.36	2.60	0.06	
	100	2.28	2.43	2.45	0.06	
Ash (g)	94	45.2	45.4	47.8	1.07	0.161
	97	46.0	45.0	49.9	1.12	
	100	43.0	46.4	46.7	1.11	
Fat (%)[2]	94	10.6	8.3	8.0	0.26	0.188
	97	10.1	8.8	7.9	0.28	
	100	11.2	9.4	8.0	0.28	
Fat (g)	94	201.7[b]	154.6[ef]	151.6[ef]	5.7	0.032
	97	191.8[bc]	167.0[de]	150.7[ef]	6.0	
	100	222.1[a]	180.7[cd]	150.7[f]	5.9	
Protein (%)[2]	94	19.31	19.87	19.86	0.16	0.167
	97	19.19	19.38	19.57	0.16	
	100	19.03	19.26	19.95	0.17	
Protein (g)	94	365.5	378.5	380.1	3.5	0.121
	97	367.7	370.6	372.0	3.7	
	100	358.6	365.2	379.0	3.7	

[a-f] Means within a row with different superscripts differ significantly ($P < 0.05$)

[1] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio

[2] Percentage of eviscerated body weight

Table 2.9. Covariate analysis[1] of carcass ash, fat and protein (% of eviscerated body weight and absolute weight) of male and female Cobb x Avian 48 broilers.

Composition Trait	Sex		Covariate		
	Female	Male	Eviscerated Body Weight[3]	SEM	Pr > F
	----------------%[4]------------------		%/g		
Ash	2.32[b]	2.53[a]	0.0002	0.03	0.0001
Fat	10.0[a]	8.3[b]	0.0018	0.16	<.0001
Protein	19.2[b]	19.8[a]	-0.0002	0.10	<.0001
	----------------g------------------		g/g		
Ash	44.1[b]	48.2[a]	0.03	0.64	<.0001
Fat	192.1[a]	156.9[b]	0.13	3.43	<.0001
Protein	364.5[b]	377.1[a]	0.19	2.12	<.0001

[a-b] Means within a row with different superscripts differ significantly ($P < 0.05$)
[1] Means adjusted for eviscerated body weight
[2] Base specifications (100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3] Slope coefficients from covariate analysis
[4] Percent of eviscerated body weight

Table 2.10. Covariate analysis[1] of carcass ash, fat and protein (% of eviscerated body weight and absolute weight) of Cobb x Avian 48 broilers in response to processing age.

Composition Trait	Processing Age (d) 42	Processing Age (d) 52	Covariate: Eviscerated Body Weight[3]	SEM	Pr > F
	----------------%[4]------------------		%/g		
Ash	2.42	2.43	0.0002	0.05	0.975
Fat	9.1	9.1	0.0018	0.22	0.984
Protein	18.57[b]	20.41[a]	-0.0002	0.13	<0.0001
	----------------g------------------		g/g		
Ash	46.15	46.14	0.03	0.86	0.996
Fat	174.2	174.8	0.13	4.62	0.942
Protein	353.9[b]	387.8[a]	0.19	2.85	<0.0001

[a-b] Means within a row with different superscripts differ significantly ($P < 0.05$)
[1] Means adjusted for eviscerated body weight
[2] Base specifications (100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3] Slope coefficients from covariate analysis
[4] Percent of eviscerated body weight

Table 2.11 Composition of *P. major* of male and female Cobb x Avian 48 broilers fed varying levels of dietary balanced protein (DBP) and metabolizable energy (ME).

		P. major	Fat (%)[1]	Protein (%)[1]	Fat (g)	Protein (g)
Effect	Level	-----g------	----------------%---------------		------------------g--------------	
DBP[2]	85	387.7	2.3	37.7	14.4	214.3
	100	414.9	2.2	38.4	13.8	225.2
	115	401.8	2.1	38.9	12.3	212.1
SEM		15.72	0.14	0.48	1.12	6.10
ME[3]	94	398.4	2.05	39.2[a]	12.5	221.2
	97	404.4	2.21	38.3[ab]	13.0	221.1
	100	401.6	2.35	37.5[b]	15.0	209.3
SEM		15.04	0.13	0.47	1.08	5.91
Sex	Female	375.5[b]	2.1	37.9	11.6[b]	201.2[b]
	Male	427.4[a]	2.3	38.8	15.4[a]	233.2[a]
SEM		12.38	0.11	0.39	0.92	5.00
Source of Variation		----------------------------------Probability--------------------------------				
DBP		0.4254	0.465	0.201	0.359	0.217
ME		0.9576	0.248	0.029	0.198	0.243
Sex		0.0024	0.151	0.120	0.002	<0.0001

[a-b] Treatment means within a column and effect with no common superscript differ significantly ($P \leq 0.05$).
[1] Percentage of *P. major*
[2] Dietary balanced protein level; 100% based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3] Metabolizable energy level; 100% based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio

Table 2.12. Covariate analysis[1] of *P. major* morphology of male and female Cobb x Avian 48 broilers in response to dietary balanced protein and metabolizable energy levels.

Effect	Level	Total Length[2]	Cranial Width[3]	Middle Width[4]	Caudal Width[5]	Cranial Thickness[6]	Middle Thickness[7]	Caudal Thickness[8]
Covariate[9]		---mm/g---						
		0.0157	0.0076	0.0146	0.0089	0.0075	0.0051	0.0021
		---mm---						
DBP[10]	85	170.93[b]	63.59	77.84[b]	51.52	25.47[b]	16.79	10.04
	100	172.48[ab]	63.59	79.85[a]	52.00	26.28[a]	17.06	9.98
	115	173.50[a]	64.40	80.34[a]	52.99	26.86[a]	17.36	10.07
SEM		0.98	2.48	0.99	2.32	0.58	0.73	1.21
ME[11]	94	171.13	63.72	78.71	52.91	25.93	17.04	10.09
	97	172.84	63.87	79.92	51.46	26.34	16.92	10.00
	100	172.94	63.99	79.41	52.14	26.34	17.25	10.01
SEM		0.99	2.48	1.00	2.33	0.59	0.74	1.21
Sex	Female	172.44	63.73	80.42[a]	52.84	26.29	17.21	9.92
	Male	172.16	64.00	78.27[b]	51.49	26.12	16.93	10.15
SEM		0.98	2.48	0.97	2.32	0.58	0.73	1.21
Age	32	165.26[c]	63.17	81.49	56.33	27.99	18.32	10.53
	42	170.75[b]	63.48	77.53	45.78	-	-	-
	52	180.90[a]	64.93	79.02	54.40	24.77	15.83	9.53
SEM		1.35	3.34	1.39	3.29	0.86	0.95	1.29
Source of Variation		---Probability---						
Body Weight at Processing		<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
DBP		0.030	0.378	0.016	0.253	0.002	0.359	0.950
ME		0.092	0.915	0.387	0.246	0.455	0.710	0.939
Sex		0.781	0.690	0.018	0.134	0.664	0.476	0.422
Age		<0.0001	0.766	0.239	0.099	0.063	0.068	0.307

[a-c] Treatment means within a column and effect with no common superscript differ significantly ($P \leq 0.05$).
[1] Means adjusted for body weight at processing
[2] Total length was measured along a diagonal axis of *P. major*, from the highest point at the cranial end of the muscle to the bottom of the keel
[3] Cranial width was measured along a horizontal axis bisecting the total length line at a point 15% below the cranial edge of the fillet. Cranial width was measured mediolaterally from the edge of the fillet closest to the keel to the outer edge of the muscle.

[4] Middle width was measured along a horizontal axis bisecting the total length line at a point 50% below the cranial edge of the fillet. Middle width was measured mediolaterally from the keel to the outer edge of the muscle.
[5] Caudal width was measured along a horizontal axis bisecting the total length line at a point 15% above the caudal edge of the fillet. Caudal width was measured mediolaterally from the keel to the outer edge of the muscle.
[6] Cranial thickness was measured at a point along the total length axis 15% below the cranial edge of the fillet.
[7] Middle thickness was measured at a point along the total length axis 50% below the cranial edge of the fillet.
[8] Caudal thickness was measured at a point along the total length axis 15% above the caudal edge of the fillet.
[9] Slope coefficients from covariate analysis
[10] Base specifications (100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[11] Base specifications (100% ME) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio

2.6 FIGURES

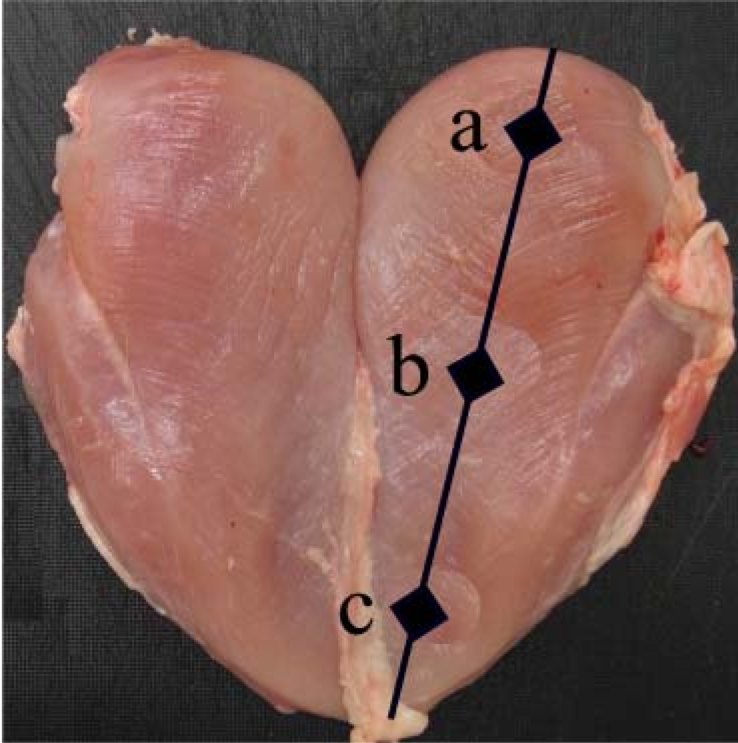

Figure 2.1. Total *P. major* length is defined along the diagonal line intersecting points a, b and c. Cranial thickness is measured at point a, 15% below the cranial edge of the fillet. Middle thickness is measured at point b, or the midpoint of the length axis. Caudal thickness is measured at point c, 15% above the caudal edge of the fillet.

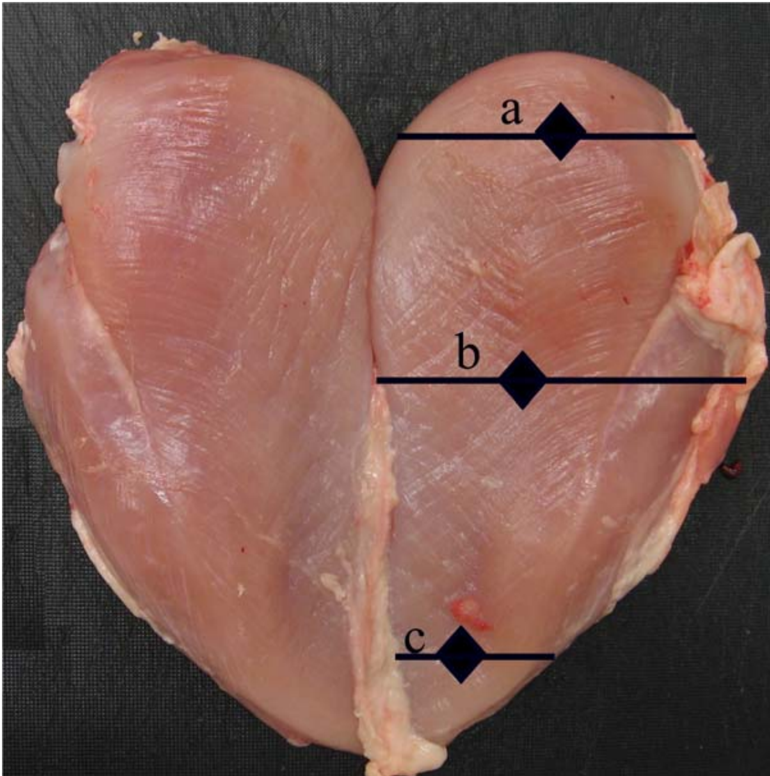

Figure 2.2. Measurements of *P. major* width. Cranial width is measured along the axis intersecting point a, 15% below the cranial edge of the fillet. Middle width is measured along the axis intersecting point b, or the midpoint fillet. Caudal width is measured along the axis intersecting point c, 15% above the caudal edge of the fillet.

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3 Effect of protein and energy level, sex and age on broiler *P. major* color and functional properties

Abstract

Color, ultimate pH (**pH$_u$**), drip and cook loss, Allo-Kramer shear (**A-K shear**), emulsification capacity and myoglobin content were assessed in Cobb-Avian 48 broilers. Chicks were randomly assigned to a 2 x 3 x 3 x 3 factorial arrangement of treatments, utilizing male and female broilers; 3 processing ages (36, 52 and 56 d); 3 metabolizable energy (**ME**) levels which were 94, 97 and 100% of Cobb-Vantress dietary specifications for maximum growth rate and feed conversion ratio (**E94, E97** and **E100)** and 3 dietary balanced protein (**DBP**) levels of 85, 100 and 115% of the Cobb-Vantress specifications for maximum growth rate and feed conversion ratio (**P85, P100** and **P115**). Experimental diets were provided in 3 phases; starter (11-21 d), grower (22-35 d) and finisher (36-56 d). Dietary balanced protein effects were limited to color traits and A-K shear while ME did not affect any of the quality traits investigated. Yellowness of *P. major* muscle was moderately correlated with cumulative corn intake (r=0.50, P<0.0001). Age at processing affected all variables except myoglobin content of *P. major* muscle. Redness of *P. major* fillets decreased with age (P<0.0001); myoglobin content had a similar decrease with age (P<0.0001). pH$_u$ decreased with age; surprisingly, higher pH (5.95) at 36 d was associated with high drip loss (3.34%). The decrease in pH with age was associated with an increase in lightness values between 36 and 52 d, with no difference between 52 and 56 d (53.15 vs 55.16 and 55.64, P<0.0001). Female *P. major* muscle was lighter than males (55.26 vs 54.05, respectively, P=0.0031) and had higher drip loss than males (3.5 vs. 3.1 %, respectively; P<0.0001), however, no sex differences were observed for pH. Modifying DBP and ME levels does not appear to adversely affect the quality of broiler *P. major*; age and sex of bird may have greater influences on functional properties than previously reported.

Keywords: dietary balanced protein, metabolizable energy, meat quality, broiler, meat color

INTRODUCTION

Over the past five decades, the poultry industry has seen tremendous changes along with a significant increase in meat consumption. As a result of the increasing demands for value-added poultry products, meat functional properties have become a more important industry issue (Barbut *et al.*, 2005). Increased yield losses make products with poor functional properties unappealing to consumers and troublesome for processors (Owens *et al.*, 2000). Recently, similar

problems associated with the pale soft and exudative (PSE) condition found in pigs were observed in poultry (Vanderwal *et al.*, 1988; Barbut, 1996; 1997b; 1998; Sosnicki *et al.*, 1998; Van Laack *et al.*, 2000; Woelfel *et al.*, 2002; Barbut *et al.*, 2005).

Feed is a significant contributor to poultry production costs. One method for reducing feed costs is least-cost diet formulation. In this scenario, nutrient requirements are met using the cheapest combination of ingredients. Feed costs can be reduced by manipulating energy and protein levels in the diet as each of these nutrients contribute significantly to the overall feed cost. The effect of modifying dietary energy and protein levels on broiler growth and performance has been well researched, however, the effect of modifying energy and protein levels on *P. major* meat color and functional properties have not received the same attention. While a low energy diet may significantly reduce feed costs, poor meat quality resulting from broilers fed those diets may outweigh the benefits.

Feed ingredient choice can affect skin and meat color; most commonly from carotenoids found in corn and corn gluten meal (Lyon *et al.*, 2004). Ingredient choice can also affect meat shear values; Lyon et al. (2004) observed that meat from broilers fed high levels of wheat (73.6%) required more force to shear than broilers fed high levels of corn (65.5%). Smith et al. (2002) observed that wheat-fed broilers had higher lightness values than corn-fed broilers, while corn-fed broilers had higher yellowness values (Smith *et al.*, 2002). Quentin et al. (2003) found that meat colour was affected by diet density; however pH at 24 h *post mortem* and drip loss were not affected. Recent research has indicated that increased dietary lysine improved *P. major* functional properties by increased pH_u and decreased drip loss (Berri *et al.*, 2008). Betti *et al.* (2009) observed that omega-3 fatty acid deposition in *P. Major* muscle associated with feeding flaxseed altered *post mortem* muscle metabolism and resulted in poor functional properties. This effect was theorized to be the result of increased development of reactive oxygen species (**ROS**). ROS have been implicated in the enzymatic regulation of glycolysis (Betti *et al.*, 2009). Lightness values of turkey meat tend to decrease with age while redness increases with age (Froning *et al.*, 1968; Mugler *et al.*, 1970). In turkeys this change has been attributed to changes in myoglobin concentration with age (Froning *et al.*, 1968). However, Smith et al. did not find color differences associated with age in broilers (2002). Further investigations are needed to clarify this relationship, especially within the range of commercial slaughter age (from 35 to 60 d in North America).

Cavitt et al. (2005) observed no sex differences for pH, lightness or Allo-Kramer shear (**A-K shear**). However, Fanatico et al. (2005) observed higher drip loss in female slow-growing

genotypes grown indoors compared to males grown under the same conditions. Berri et al. (2007) found that males had higher lightness values and drip loss compared to female.

Strong positive correlations between emulsification capacity (**EC**) and water-holding capacity (**WHC**), EC and pH have been found by Qiao et al. (2001). The effect of dietary protein and energy levels on EC has not been investigated. Emulsification capacity is affected by protein solubility and pH (Kurt and Zorba, 2005). Low pH is associated with poor protein solubility and EC (Zorba *et al.*, 1995; Kurt and Zorba, 2005).

Lightness is commonly proposed as a screening tool to detect PSE-like poultry meat; several scientists have observed strong correlations between lightness, pH and WHC (Barbut, 1993; 1996; McCurdy *et al.*, 1996; 1997b; Owens *et al.*, 2000; Woelfel *et al.*, 2002).

The present experiment compared color and functional properties from broilers fed graded levels of dietary protein and energy. The effects of sex of birds, age and interactions with protein and energy level were also measured.

3.1 MATERIALS & METHODS

3.1.1 Experimental Design

The present experiment is an extension of a larger project investigating the impact of dietary balanced protein (**DBP)** and metabolizable energy (**ME**) levels on broiler performance, yield and carcass composition, with a 2 x 2 x 3 x 5 factorial treatment arrangement (Zuidhof *et al.*, 2008). The project utilized male and female broilers; 2 levels of early nutrition (0 – 10 d); and after 11 d, 3 levels of ME which were 94, 97 and 100% of Cobb-Vantress ME recommendations for maximum growth rate and feed conversion ratio (**E94**, **E97** and **E100**, respectively). There were 5 DBP levels: 85, 92.5, 100, 107.5 and 115% of Cobb-Vantress protein recommendations for maximum growth rate and feed conversion ratio, balanced for 6 amino acids: Methionine, Methionine + Cysteine, Tryptophan, Threonine, and Arginine (**Met, Met+Cys, Trp, Thr** and **Arg**). Recommended diet specifications (100% level for ME and DBP) are presented in Table 3.1.

Functional properties and color of *P. major* were measured on a subset of birds and consisted of a 2 x 3 x 3 x 3 factorial arrangement of treatments, with male and female broilers, 3 ME levels (94, 97 and 100%); 3 DBP levels: 85, 100 and 115% of recommended (**P85**, **P100** and **P115**, respectively); and 3 processing ages (36, 52 and 56 d).

3.1.2 Experimental Diets

To minimize variation in experimental diets due to ingredient variability, four basal diets per phase were formulated (Table 3.2), and then blended (Table 3.3) to make the 9 treatment diets

for the starter (11 – 21 d), grower (22-35 d) and finisher (36 to 56 d) phases. The ingredient composition of all experimental diets is presented in Table 3.4. The crude protein and amino acid content of the major dietary ingredients was measured before the formulation of the four basal diets (Zuidhof *et al.*, 2008). Diets were provided in pellet form.

Crude protein content (Leco Truspec CN3402, LECO Corporation., St. Joseph, MI 49085), gross energy (IKA Calorimeter C 5000, IKA., Werke Staufen, Germany), amino acids (Sedgwick *et al.*, 1991), dry matter and ash (AOAC, 2006) was measured for all diets in all phases. Apparent metabolizable energy (**AME**) was measured for each diet (Scott and Boldaji, 1997; Scott and Hall, 1998). AME values for grower diets A, C and D were 4.3, 5.6 and 6.0% lower than targets, possibly due to variation in ingredient ME levels (Zuidhof *et al.*, 2008). Ingredient ME levels were not evaluated prior to formulation (Zuidhof *et al.*, 2008). Expected and actual protein and ME contents of the experimental diets are presented in Table 3.5.

3.1.3 Stocks and Management

The Canadian Council of Animal Care (1993) guidelines for animal care were met throughout the duration of the experiment and all protocols were approved by the University of Alberta Animal Care and Use Committee – Livestock. Chicks were feather sexed and identified with neck tags (Heartland Animal Health, Fair Play, MO 65649) at hatch prior to placement. Birds were housed sex-separately throughout the experiment. During the early nutrition phase (0-11 d), 3424 Cobb x Avian 48 broiler chicks were randomly distributed among 16 floor pens. At 12 d birds were distributed to 60 floor pens. Prestarter nutrition treatments were nested within pen after the early nutrition phase. A 23L:1D lighting schedule was provided for the first 3 d followed by 20L:4D to 56 d. Access to feed and water (nipple drinkers) was provided *ad libitum* for the duration of the experiment. Feed intake data was collected weekly and cumulative corn intake (grams per bird) was calculated.

3.1.4 Processing and Sample Preparation

Eight birds per DBP x ME x Sex interaction were processed at 36, 52 and 56 d. Birds were crated for a 12 h feed withdrawal period within the rearing barn and were transported to the processing facility immediately prior to slaughter. The birds were electrically stunned, slaughtered by a ventral neck cut and bled for 2 minutes. After scalding (63°C) for 45 s, carcasses were mechanically defeathered. Carcasses were manually eviscerated and held in an air chiller at 4°C for 24 h prior to deboning. Sex was confirmed during evisceration.

3.1.4.1 Color

A Minolta CR-400 (Konica Minolta Sensing Americas, Inc, Ramsey, NJ 07446) colorimeter using illuminant D65 as the light source was used to assess the color (CIE L* a* b*)

of *P. major* muscle. L* refers to lightness, a* refers to redness and b* refers to yellowness. Color was measured at deboning and at 24 h *post mortem* on the cranial, medial surface (bone side) of individual *P. major* fillets in an area free from obvious color defects (bruises, blood spots, or surface discolorations). Three readings per fillet were taken and the average reading was recorded. Hue angle, ($\tan^{-1}(b/a)$), measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006). As hue angle increases, visually perceived redness decreases (Little, 1975; Brewer *et al.*, 2006). Chroma ($(a^2+b^2)^{1/2}$) refers to the brightness/colorfulness of an object (Fairchild, 2004).

3.1.4.2 Ultimate pH.

Ultimate pH (**pH_u**) was measured via direct insertion of a Hanna Instruments (Woonsocket, RI, 02895) electrode to each fillet at 24 h *post mortem*. An incision 0.5 to 1 cm deep was made to allow insertion of the electrode.

3.1.4.3 Water Holding Capacity.

A core sample (25 x 50 mm) was excised from the upper portion of the *P. major* fillet and weighed to assess WHC. The core sample was suspended on cheese cloth for 48 h in a sealed plastic container and reweighed. Drip loss is expressed as a percentage of the initial weight of the core sample. Cooking loss was evaluated by cooking at 163ºC in a convection oven until an internal temperature of 80°C was reached (Digi-Sense RK-92000 Benchtop 115V, Cole Parmer Instrument Co, Montreal, QC H4P 2R9). The samples were cooled to room temperature and reweighed to determine cooking loss (expressed as a percentage of initial weight of the core sample).

3.1.4.4 Allo-Kramer Shear Determination.

Following cooking loss determination, A-K shear values were measured using an Instron Universal Testing Machine (Model 4411, Instron Corp., Canton, MA) equipped with an Allo-Kramer cell. The same sample used for cooking loss determination was placed with the blades at a right angle to the muscle fibers using a 200-kg load cell and cross head speed of 100 mm/min. Shear values are reported as kilograms per gram.

3.1.4.5 Emulsification Capacity and Myoglobin Content.

Following pH, colour, and WHC determination, the fillets were ground using an industrial blender. Emulsification capacity was evaluated at 6 to 7 d *post mortem* using the method described by Qiao et. al. (2001). Ground samples for EC analysis were stored at 4°C prior to analysis. Myoglobin content was measured using the methods described by Kryzwicki (1982) and Kannan et. al. (2001). Ground samples were stored at -20°C for 6 mo prior to analysis.

3.1.5 Statistical Analysis

Data were analyzed as a 4-way analysis of variance using the mixed model procedure of SAS® (SAS Institute Inc., 2008).

Y_{ijklm}=μ + P_i + E_j + PE_{ij} + S_k + SP_{ik} + SE_{jk} + A_l + AP_{il} +AE_{jl} + AS_{kl} + + ε_{ijklm}

Where Y_{ijklm}= variable measured for the mth bird, μ = overall mean, P_i = effect of the ith DBP level; E = effect of the jth ME level; PE_{ij} =interaction between DBP and ME; S_k = effect of the kth sex; SP_{ik} = interaction between sex and DBP; SE_{jk} = interaction between sex and ME; A_l = effect of lth age; AP_{il} = interaction between age and DBP; AE_{jl} = interaction between age and ME; AS_{kl} = interaction between sex and age; and ε_{ijklm} = residual error component.

Differences between least squares means were determined using pairwise comparisons, and significance was determined at the P≤0.05 level. Pearson's correlations were determined to identify relationships between meat quality parameters. Emulsification capacity and myoglobin data were analyzed with bird as a random effect to account for within bird variation. Emulsification capacity was analyzed with day of analysis within age as a random term to account for differences due to date of analysis. The SEM for the group with the least number of birds was reported.

3.2 RESULTS AND DISCUSSION

3.2.1 pH

Dietary Balanced Protein and ME did not affect pH_u (P=0.233 and P=0.731, respectively; Table 3.6, Table 3.7). Ultimate pH was highest at 36 d, with no difference between 52 and 56 d (5.95 vs 5.86 and 5.84, respectively; Table 3.8). Similar results have been reported previously (Sandercock *et al.*, 2001). An interaction was also observed between age and DBP (Figure 3.1). At 52 and 56 d, there were no differences in pH_u due to DBP level; at 36 d pH_u in the P100 treatment was higher than P115 while P85 was not different from either. Berri *et al.* (2008) observed that feeding graded levels of dietary lysine from 0.94 to 1.25% in finisher diets to 42 d resulted in higher pH_u. The present study included lysine at similar proportions (0.94 to 1.27%) from 36 to 56 d; the highest lysine level was present in the P115 treatment. However, the P100 treatment resulted in higher pH_u at 36 d compared to the P115 treatments. Berri *et al* (2008) maintained constant amounts of Met, Met+Cys, Thr and Trp while the present study maintained constant ratios between lysine and Met, Met+Cys, Thr, Trp and Arg. The effect of lysine on pH_u observed by Berri *et al* (2008) may be related to this difference.

Ultimate pH was also affected by an interaction between age and sex (Figure 3.2). At 56 d pH_u was lower in females than males, at all other ages there were no differences between the sexes. Acidification *post mortem* is the result of lactic acid production from glycolytic

metabolism. Differences due to sex indicate that muscle metabolism differed between males and females. Currently there is limited information regarding the effect of sex on broiler *P. major* muscle glycogen content or pH and their influence on the development of meat with PSE characteristics. (Ngoka *et al.*, 1982) reported no effects of sex on *P. major* muscle pH, WHC, cooking loss, and CIE L* a* b* attributes in turkeys. Barbut (1997a) and McCurdy *et al.* (1996) noted that *P. major* from mature turkey hens exhibited higher average L* values than those from young toms (48.9 vs 44.7). The reasons for these differences are not known but they could be due to age or sex related effects on muscle color. Wheeler *et al.* (1999) observed no significant differences in *P. major* muscle pH and L* values between sexes. Boulianne and King (1995) indicated that sex could play a potential role in predisposing chicken breast meat to PSE. Research in rabbits has revealed higher glycolytic enzyme levels (aldolase) in females in some muscles, which would indicate a greater potential for *post mortem* acidification; however, no differences in meat quality were observed (Dalle Zotte *et al.*, 1996). Several authors have reported on the effects of sex on the incidence of PSE in pork carcasses (Park, 1980; 1985; Choi *et al.*, 1998). Park *et al.* (1980) reported that female hog carcasses showed a higher incidence of PSE than males (35.1 % vs 30.3 %). In a subsequent study, these researchers observed a higher incidence of PSE in females than in males and attributed the disparity to differences in backfat thickness (Park, 1985).

Sibut *et al*. (2008) observed meat quality differences between strains of broiler chickens genetically selected for fatness or leanness. Glycogen content and glycolytic potential were higher in the fat line, which subsequently resulted in lower pH_u and higher drip loss and lightness values (Sibut *et al.*, 2008). Adenosine monophosphate activated protein kinase (**AMPK**) was higher in lean birds at 15 min *post mortem*, possibly due to the lower level of glycogen (Sibut *et al.*, 2008). AMPK is involved in the regulation of cellular energy supplies; AMPK switches on ATP-generating pathways while stopping ATP-utilizing pathways (Proszkowiec-Weglarz *et al.*, 2006; Richter and Ruderman, 2009). AMPK has been implicated in increased rates of glycolysis and lactic acid formation *post mortem* (Shen *et al.*, 2007). Sibut *et al*. (2008) observed that AMPK gene expression differed between the fat and lean broiler strains and further noted that the gene encoding for a portion of the AMPK complex is located near a quantitative trait locus that has been identified (Demeure *et al.*, 2004) to influence fatness in pigs. It has been well established that turkeys and broiler females have a higher proportion of fat than males (Summers *et al.*, 1965; Jackson *et al.*, 1982; Havenstein *et al.*, 1994; Gous *et al.*, 1999; Havenstein *et al.*, 2003; Latshaw and Moritz, 2009). Based on the preceding information, differences in pH and other functional properties between sexes may be attributed to high AMPK activity *post mortem*,

higher glycogen content as the result of higher fat content or a combination of high AMPK and glycogen content. Further research is required to determine the full extent of AMPK on *post mortem* metabolism in broilers.

3.2.2 Water Holding Capacity

Drip loss was lowest at 56 d (3.17%) while no differences were observed between 36 and 52 d (3.34 and 3.36%, respectively; Table 3.8). Cook loss decreased with age (25.61 vs. 23.71 vs. 22.24%, respectively; Table 3.8). These results seem to be counter-intuitive since pH_u was higher at 36 d, typically low pH_u is associated with lower WHC (Briskey, 1964; Warriss, 2000; Alvarado and Sams, 2004). Drip loss was also higher in females than males (3.50 vs 3.08%, respectively; Table 3.9); however, this result cannot be attributed to differences in pH_u. This in contrast to results reported by Berri et al. (2007); in that study males had lighter *P. major* and higher drip loss.

Studies relating changes in water holding properties with age at slaughter have been well documented in other species; however research in poultry has been limited. Northcutt *et al.* (1994) reported an age related change in the ability of *P. major* muscle to hold water; *P. major* from younger broilers (21 d) had higher rates and initial amounts of drip loss than meat from older broilers (28, 35 and 42 d). They indicated that these changes could be the result of alterations in muscle protein isoforms that occur during maturation. Ngoka and Froning (1982) reported no significant differences in WHC and cooking losses between *P. major* muscles of turkeys of 16 and 20 wk of age. However, the 16 wk old turkeys had a significantly higher thaw loss than the 20 wk old turkeys.

3.2.3 Color

3.2.3.1 Lightness

Metabolizable energy level did not affect any color properties (Table 3.7). Color attributes of *P. major* muscle differed between sexes (Table 3.9). Muscle from female birds exhibited higher L* values than those of males (55.30 and 53.91, respectively; Table 3.9). These differences appeared to be the result of a higher degree of protein denaturation in female muscles that exhibited lower pH_u. Lighter meat is often associated with decreased WHC which was also observed in females in the present study. Furthermore, lightness increased with age between 36 and 52 d, there was no difference between 52 and 56 d lightness values (53.15 vs 55.16 and 55.64, respectively; Table 3.8). These results disagree with Smith *et al.* (2002), who observed no differences in *P. major* color as broilers aged from 42 to 52 d. It should be noted that increases in lightness with age were associated with decreases in pH in the current study (Table 3.8).

3.2.3.2 Redness

Redness values were highest at 36 d with no differences between 52 and 56 d (3.75 vs 3.49 and 3.27, respectively; Table 3.8). Redness is normally associated with myoglobin content of the muscle. In contrast to the present study, Fletcher (2002) theorized that *P. major* muscle becomes darker and more red with age due to an increase in muscle myoglobin.

3.2.3.3 Yellowness

As DBP decreased from 115 to 85%, *P. major* meat yellowness values increased (4.13, 5.63 and 6.96, respectively; Table 3.6). In order to achieve the various protein:energy ratios, some of the diets contained up to 50% corn (Table 3.4). It was hypothesized that dietary corn affected yellowness values; yellowness and cumulative corn intake demonstrate a moderate level of correlation (r=0.50, Table 3.10).

Cumulative corn intake was included as a covariate in a mixed model analysis of covariance but was not found to be significant, likely due to high variation between birds with similar corn intake (Figure 3.3). Earlier research indicated that diets high in corn (70%) resulted in yellower meat than high wheat (74%) diets (Lyon *et al.*, 2004). In the present experiment, the highest level of corn in the diet was 52% (Table 3.4), which was apparently not high enough to consistently affect the color of *P. major* meat. Visual observations of skin and whole carcasses indicated that birds fed high corn diets were more yellow. Yellowness increased between 36 and 52 d; there was no difference between 52 and 56 d (4.45 vs. 6.14 and 6.12, respectively; Table 3.8). This difference may be attributed to an increase in corn intake with age (Figure 3.3).

3.2.3.4 Hue Angle and Chroma

Hue angle and chroma were not affected by ME level (Table 3.7). Hue angle decreased as DBP level increased indicating that meat appeared less red (62.56 vs. 58.31 vs. 47.88 respectively; Table 3.6). Meat appeared brighter (increasing chroma values) as DBP level decreased (2.76 vs. 2.98 vs. 3.97, respectively; Table 3.6). An interaction between age and sex for chroma was observed (Figure 3.4). At 36 d of age, females had lower chroma values than males, while males had lower chroma values at 52 and 56 d.

3.2.4 Allo-Kramer Shear

The P115 treatment (4.16 kg/g) had higher A-K shear values than the P100 treatment (3.92 kg/g) while the P85 was not different from either (4.04 kg/g; Table 3.6). Allo-Kramer shear values decreased with age between 36 and 52 d, with no differences observed between 52 and 56 d (3.80 vs 4.11 and 4.22 kg /g, respectively; Table 3.8). Several studies that measured tenderness at various ages between 5 to 16 wk have observed decreases in tenderness as bird age increased (Awonorin and Ayoade, 1992; Poole *et al.*, 1999b; Northcutt *et al.*, 2001). Decreased tenderness

has been associated with increased cook losses (Betti *et al.*, 2009); however, cook loss in the present study decreased with age and no effects of DBP on cook loss were observed.
Fibre diameter is often implicated in differences in meat tenderness since it has been reported to increase with age (Dransfield and Sosnicki, 1999). Decreases in tenderness associated with age have also been attributed to an increase in insolubility and crosslinkages between collagen fibres (Iqbal *et al.*, 1999; Aberle *et al.*, 2001; Fletcher, 2002; Davies, 2004; Fanatico *et al.*, 2005). Increased crosslinkages are theorized to decrease tenderness due to tension created from connective tissue shrinkage and subsequent moisture losses during cooking (Kurth, 1993; Lepetit, 2008). Although the observed tenderness differences are significant, they are below 6.0 kg/g; A-K shear values below this value are considered 'tender' to 'very tender' in sensory panel testing (Lyon and Lyon, 1990).
No ME effects were observed on A-K shear values, in agreement with several previous studies that reported no differences in *P. major* meat tenderness were observed when dietary energy was modified (Goodwin *et al.*, 1969; Arafa *et al.*, 1985; Sonaiya *et al.*, 1990).

3.2.5 Emulsification Capacity

Emulsification capacity is the defined as the amount of oil that can be emulsified (suspended) in protein solution (Kurt and Zorba, 2005). Emulsification capacity is affected by protein solubility; when pH is close to the isoelectric point EC will be decreased (Zorba *et al.*, 1995). Emulsification capacity varied with age; the lowest EC was observed at 56 d (56.62 mL), followed by 58.16 mL at 36 d with the highest EC observed at 52 d (58.47 mL). Qiao *et al*. (2001) observed higher EC values than the present study. Emulsification capacity was measured at 6 and 7 d *post mortem* in the present study, storage of the samples may have compromised the result.
Although an interaction between sex and ME level was observed for EC, the difference between high and low values was approximately 0.5 mL (Figure 3.5). The differences observed in the current study, while statistically significant, are likely not of commercial importance.

3.2.6 Myoglobin Content

Meat color is predominantly influenced by the presence of hemoglobin and myoglobin in the muscle (Warriss, 2000; Barbut, 2002). Studies in turkeys and other species have indicated that myoglobin content increases with age (Froning *et al.*, 1968; Fletcher, 1999b); this effect was not observed in the present study. Myoglobin content of *P. major* was not different at any age (P=0.6120; Table 3.8). Broiler *P. major* is largely composed of Type IIb white muscle fibres (Smith and Fletcher, 1988; Taylor, 2004); these fibres utilize anaerobic metabolism and have less myoglobin and capillaries which accounts for their lighter, less red color (McKee, 2003).

3.2.7 Pearson's Correlations

Results of the present experiment did not show strong correlations between lightness, pH_{24} and WHC (Table 3.10). This disagrees with several previous studies that reported strong correlations between lightness, pH and WHC (Barbut, 1993; 1996; 1997b; Woelfel *et al.*, 2002). Many of these studies have classified meat as pale, normal or dark and as such the correlation between traits may be exaggerated. When populations are randomly sampled, as in the current experiment, the correlations between lightness, pH and WHC may not be as strong. Others have suggested that the relationship between lightness and pH may not be linear (Owens *et al.*, 2000; Fraqueza *et al.*, 2006). Further studies should investigate relationships between *P. major* meat quality parameters on randomly selected samples.

3.3 CONCLUSIONS

Although meat quality parameters were affected by modifying dietary protein and energy levels, it appears that none of the changes resulted in an increase in meat quality problems such as PSE-like meat. This is quite similar to the conclusions of Quentin et al (2003). Sex-related differences in *post mortem* metabolism may be the reason for observed differences in pH, color and WHC. Further research that characterizes age and sex-related differences in functional properties could improve the efficiency of the broiler supply chain. As such, modifying protein and energy levels to improve the profitability of the broiler supply chain should not have major implications for product quality.

3.4 TABLES

Table 3.1. Nutrient composition of the 100%[1] Energy, 100%[1] DBP diets for prestarter (0-10 d), starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

Calculated nutrient composition	Prestarter HighPS[2]	Prestarter LowPS[2]	Starter	Grower	Finisher
ME (kcal/kg)	3,150	2,976	3,150	3,200	3,250
Protein (%)	22.5	21	22.50	20.00	19.00
Arginine-total (%)	1.43	1.26	1.40	1.30	1.20
Lysine-total (%)	1.35	1.20	1.40	1.20	1.10
Methionine-total (%)	0.55	0.49	0.60	0.60	0.50
M+C-total (%)	0.99	0.89	1.00	0.90	0.90
Tryptophan-total (%)	0.22	0.19	0.30	0.30	0.30
Threonine-total (%)	0.89	0.79	0.90	0.80	0.80
Calcium (%)	0.90	0.90	0.90	0.90	0.80
Phosphorous Available (%)	0.45	0.45	0.50	0.40	0.40
Sodium (%)	0.20	0.20	0.20	0.20	0.20
Chloride (%)	0.20	0.20	0.50	0.40	0.40
Potassium (%)	0.65	0.65	1.00	0.90	0.80

Table modified from Zuidhof, M. J., F. I. L. Hernandez, D. R. Korver, and R. A. Renema. 2008. An integrated nonlinear analysis of nutritional effects on broiler performance. Pages 29-63 *in* Final Report to Funders: Value-based marketing decision support through characterization of growth, fatness and yield. Alberta Agriculture and Rural Development, Edmonton, AB.

[1] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2]HighPS: - prestarter high; LowPS – prestarter low.

Table 3.2. Nutritional composition of formulated diets used to blend 9 treatment diets for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases for 85, 100 and 115%[1] dietary balanced protein (DBP) and 94, 97 and 100%[1] Metabolizable Energy (ME) combinations.

	Starter				Grower				Finisher			
Item	A[2]	B[3]	C[4]	D[5]	A[2]	B[3]	C[4]	D[5]	A[2]	B[3]	C[4]	D[5]
Nutrient composition	Calculated											
ME (kcal/kg)	2,961	3,150	2,961	3,150	3,008	3,200	3,008	3,200	3,055	3,250	3,055	3,250
Protein (%)	19.13	19.13	25.88	25.88	17.00	17.00	23.00	23.00	16.15	16.15	21.85	21.85
Fat (%)	3.04	5.42	1.98	4.30	3.93	6.97	3.11	6.18	4.69	7.00	3.86	6.50
Fiber (%)	5.25	4.51	5.22	4.40	5.15	5.06	5.24	5.18	5.13	4.22	5.19	4.61
Arginine-total (%)	1.22	1.22	1.64	1.64	1.10	1.10	1.48	1.48	0.99	0.99	1.35	1.35
Lysine (%)	1.15	1.15	1.55	1.55	1.02	1.02	1.38	1.38	0.94	0.94	1.27	1.27
Methionine-total (%)	0.49	0.49	0.68	0.68	0.47	0.47	0.66	0.66	0.45	0.45	0.63	0.63
M+C-total (%)	0.84	0.84	1.14	1.14	0.80	0.80	1.08	1.08	0.77	0.77	1.04	1.04
Threonine-total (%)	0.76	0.76	1.02	1.02	0.70	0.70	0.94	0.94	0.64	0.64	0.86	0.86
Tryptophan-total (%)	0.27	0.26	0.38	0.37	0.24	0.24	0.33	0.33	0.23	0.22	0.32	0.31
Calcium (%)	0.90	0.90	0.90	0.90	0.88	0.88	0.88	0.88	0.84	0.84	0.84	0.84
Phosphorous												
Available (%)	0.45	0.45	0.45	0.45	0.42	0.42	0.42	0.42	0.40	0.40	0.40	0.40
Sodium (%)	0.23	0.23	0.23	0.23	0.20	0.20	0.20	0.20	0.19	0.19	0.19	0.19
Chloride (%)	0.47	0.47	0.49	0.48	0.43	0.43	0.44	0.44	0.41	0.41	0.43	0.42
Potassium (%)	0.98	0.90	1.12	1.06	0.88	0.85	1.02	1.00	0.86	0.77	0.98	0.93
Choline (mg/kg)	1490	1425	1533	1522	1373	1297	1427	1356	1356	1297	1370	1343
Vitamin E (IU/kg)	64	63	60	59	65	63	61	60	65	64	61	61
Nutrient composition	Analyzed											
ME (kcal/kg)	3,009	3,169	3,014	3,094	2,909	3,119	2,842	3,008	3,022	3,137	2,972	3,171
Protein (%)	20.70	19.80	27.80	27.80	16.39	17.41	23.41	22.31	16.09	16.78	20.76	21.52
Arginine-total (%)	-	-	-	-	1.12	1.10	1.24	1.34	1.06	1.02	1.34	1.36
Lysine-total (%)	-	-	-	-	1.15	1.13	1.12	1.09	0.90	1.26	1.34	1.28
Methionine-total (%)	-	-	-	-	0.54	0.42	0.66	0.63	0.45	0.44	0.66	0.64
Threonine-total (%)	-	-	-	-	0.72	0.71	0.77	0.77	0.62	0.67	0.81	0.72

Table modified from Zuidhof, M. J., F. I. L. Hernandez, D. R. Korver, and R. A. Renema. 2008. An integrated nonlinear analysis of nutritional effects on broiler performance. Pages 29-63 *in* Final Report to Funders: Value-based marketing decision support through characterization of growth, fatness and yield. Alberta Agriculture and Rural Development, Edmonton, AB.

[1]Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2]Diet A: 85% of base DBP; 94% base ME.
[3]Diet B: 85% of base DBP; 100% base ME.
[4]Diet C: 115% of base DBP; 94% base ME.
[5]Diet D: 115% of base DBP; 100% base ME.

Table 3.3. Blending[1] specifications for 9 experimental diets with combinations of 94, 97 or 100%[2] metabolizable energy (ME) and 85, 100 or 115%[2] dietary balanced protein (DBP) for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

	DBP Level (%)		
ME Level (%)	85	100	115
94	A[3]	AC	C[5]
97	AB	ABCD	CD
100	B[4]	BD	D[6]

[1] For each phase, diets A, B, C, and D were formulated. Each instance of A, B, C or D indicates than an equal amount of that diet was present in the experimental diet.
[2] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio
[3]Diet A: 85% of base DBP; 94% base ME.
[4]Diet B: 85% of base DBP; 100% base ME.
[5]Diet C: 115% of base DBP; 94% base ME.
[6]Diet D: 115% of base DBP; 100% base ME.

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Table 3.4. Ingredient composition of experimental diets containing combinations of 94, 97 or 100%[1] metabolizable energy (ME) and 85, 100 or 115%[1] dietary balanced protein (DBP) for starter (11-21 d), grower (22-35 d) and finisher (36-56 d) phases.

ME (%)	Phase	DBP (%)	Corn	Wheat	Soybean meal	Wheat bran	Canola oil	DL-Met	L-Lys HCL	L-Thr	Arg	Dicalcium phosphate	Calcium carbonate	Sodium chloride	Cocci-diostat	Anti-biotic	Pre-mix[2]
			---------								%---------						
94	Starter	85	47.0	0.0	22.0	25.0	0.35	0.16	0.25	0.01	0.02	1.26	1.36	0.56	0.05	0.05	0.50
		100	23.5	19.1	26.8	24.5	0.32	0.23	0.29	0.03	0.01	1.19	1.38	0.56	0.05	0.05	0.50
		115	0.0	38.3	31.6	24.1	0.28	0.29	0.33	0.05	0.00	1.13	1.41	0.56	0.05	0.05	0.50
	Grower	85	50.0	2.0	16.2	25.0	1.14	0.18	0.28	0.05	0.08	1.13	1.41	0.48	0.05	0.05	0.50
		100	30.0	17.5	20.7	25.0	1.16	0.25	0.32	0.07	0.07	1.07	1.43	0.49	0.05	0.05	0.50
		115	10.0	32.9	25.1	25.0	1.17	0.31	0.35	0.10	0.06	1.01	1.45	0.49	0.05	0.05	0.50
	Finisher	85	52.8	0.0	15.1	25.0	1.84	0.16	0.22	0.01	0.02	1.04	1.36	0.46	0.05	0.05	0.50
		100	31.3	17.7	18.8	25.0	1.87	0.23	0.26	0.04	0.01	0.98	1.39	0.46	0.05	0.05	0.50
		115	9.8	35.3	22.6	25.0	1.90	0.29	0.29	0.06	0.00	0.92	1.42	0.46	0.05	0.05	0.50
97	Starter	85	43.5	5.0	21.8	22.4	1.67	0.16	0.26	0.01	0.03	1.31	1.34	0.56	0.05	0.05	0.50
		100	21.8	22.3	26.9	21.7	1.60	0.23	0.29	0.03	0.01	1.25	1.36	0.56	0.05	0.05	0.50
		115	0.0	39.6	32.1	21.0	1.53	0.29	0.33	0.05	0.00	1.19	1.38	0.56	0.05	0.05	0.50
	Grower	85	44.7	6.0	15.8	25.0	2.79	0.18	0.30	0.06	0.08	1.14	1.42	0.48	0.05	0.05	0.50
		100	24.9	21.2	20.3	25.1	2.80	0.25	0.33	0.08	0.08	1.07	1.44	0.49	0.05	0.05	0.50
		115	5.0	36.5	24.8	25.1	2.82	0.32	0.36	0.10	0.07	1.01	1.46	0.49	0.05	0.05	0.50
	Finisher	85	49.9	5.0	14.9	21.6	3.13	0.16	0.23	0.02	0.03	1.10	1.33	0.46	0.05	0.05	0.50
		100	29.2	21.1	18.9	22.3	3.22	0.23	0.26	0.04	0.02	1.03	1.36	0.46	0.05	0.05	0.50
		115	8.4	37.1	22.9	22.9	3.32	0.29	0.29	0.06	0.00	0.96	1.40	0.47	0.05	0.05	0.50
100	Starter	85	40.0	10.0	21.5	19.7	3.00	0.17	0.27	0.02	0.04	1.35	1.33	0.56	0.05	0.05	0.50
		100	20.0	25.5	27.1	18.8	2.89	0.23	0.29	0.04	0.02	1.30	1.34	0.56	0.05	0.05	0.50
		115	0.0	41.0	32.6	17.8	2.77	0.30	0.32	0.05	0.00	1.25	1.35	0.56	0.05	0.05	0.50
	Grower	85	39.4	10.0	15.4	25.0	4.43	0.19	0.31	0.06	0.09	1.14	1.42	0.49	0.05	0.05	0.50
		100	19.7	25.0	20.0	25.1	4.45	0.25	0.34	0.09	0.08	1.08	1.45	0.49	0.05	0.05	0.50
		115	0.0	40.0	24.5	25.2	4.47	0.32	0.37	0.11	0.07	1.01	1.47	0.49	0.05	0.05	0.50
	Finisher	85	47.1	10.0	14.8	18.3	4.43	0.16	0.24	0.03	0.04	1.16	1.31	0.46	0.05	0.05	0.50
		100	27.0	24.5	19.0	19.5	4.58	0.23	0.26	0.04	0.02	1.08	1.34	0.46	0.05	0.05	0.50
		115	6.9	38.9	23.2	20.8	4.73	0.30	0.29	0.06	0.00	1.00	1.38	0.47	0.05	0.05	0.50

[1] Base specifications (100% ME and 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.

[2] Premix provided the following (per kg of diet): vitamin E, 3 g; lignosulfonate binder, 6 g; choline chloride, 5 g (provided 400 mg) and broiler premix, 5 g containing: vitamin A (as retinyl acetate), 10,000 IU; vitamin D_3 (as cholecalciferol), 2,500 IU; vitamin K (menadione sodium bisulfite), 2.0 mg; vitamin E (as D-α-tocopherol), 35 IU; biotin, 0.18 mg; folic acid , 0.8 mg; niacin, 65 mg; D-pantothenic acid, 14 mg; pyridoxine, 4 mg ; riboflavin, 5 mg; thiamine, 2 mg; vitamin B_{12}, 20 µg; iron, 100 mg, Mn, 70 mg; Cu 8.5 mg; Se, 0.10 mg; zn, 80 mg; I, 0.5 mg, 5

Table 3.5. Expected and actual protein and energy composition of broiler starter (11-21 d), grower (22-35 d) and finisher (36-56 d) experimental diets varying in dietary balanced protein and metabolizable energy.

	Dietary Balanced Protein (% of recommended)[1]																	
	85						100						115					
	Starter		Grower		Finisher		Starter		Grower		Finisher		Starter		Grower		Finisher	
ME[1] Level	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual	Expected	Actual
	Dietary Energy Level (kcal/kg)																	
94	2964	3009	3011	2909	3058	3022	2964	3012	3011	2876	3058	2997	2964	3014	3011	2842	3058	2972
97	3056	3089	3104	3014	3153	3080	3056	3072	3104	2970	3153	3076	3056	3054	3104	2925	3153	3072
100	3150	3169	3200	3119	3250	3137	3150	3132	3200	3064	3250	3154	3150	3094	3200	3008	3250	3171
	Protein Level (%)																	
94	19.1	20.7	17.0	16.4	16.2	16.1	22.5	24.3	20.0	19.9	19.0	18.4	25.9	27.8	23.0	23.4	21.9	20.8
97	19.1	20.3	17.0	16.9	16.2	16.4	22.5	24.0	20.0	19.9	19.0	18.8	25.9	27.8	23.0	22.9	21.9	21.1
100	19.1	19.8	17.0	17.4	16.2	16.8	22.5	23.8	20.0	19.9	19.0	19.2	25.9	27.8	23.0	22.3	21.9	21.5

[1] Base specifications (100% ME, 100% DBP) are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio

Table 3.6. Effect of dietary balanced protein level (DBP) on *P. major* muscle lightness, redness, yellowness, hue, chroma, drip and cooking losses, emulsification capacity, myoglobin content and Allo-Kramer shear values in Cobb x Avian 48 broilers.

	Dietary Balanced Protein (% of recommended)				
Quality Trait	85	100	115	SEM	Pr > F
Lightness	53.71[b]	54.72[a]	55.52[a]	0.30	0.0027
Redness	3.61	3.32	3.58	0.10	0.0808
Yellowness	6.96[a]	5.63[b]	4.13[c]	0.11	<.0001
Hue Angle[1]	62.56[a]	58.31[b]	47.88[c]	0.81	<.0001
Chroma[2]	3.27[a]	2.98[b]	2.76[c]	0.03	<.0001
pH_u	5.89	5.90	5.87	0.01	0.2333
Driploss (%)	3.21	3.27	3.39	0.05	0.0763
Cookloss (%)	23.86	23.61	24.08	0.27	0.4703
Myoglobin (mg/g)	0.18	0.19	0.23	0.02	0.2591
Emulsification Capacity (ml oil)	57.79	57.74	57.72	0.08	0.8445
Allo-Kramer Shear (kg /g)	4.04[ab]	3.92[b]	4.16[a]	0.06	0.0358

[a-c] Means within a row with different superscripts differ significantly ($p \leq 0.05$).
[1] DBP levels are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 3.7. Effect of metabolizable energy (ME) level on *P. major* muscle lightness, redness, yellowness, hue, chroma, drip and cooking losses, emulsification capacity, myoglobin content and Allo-Kramer shear values in Cobb x Avian 48 broilers.

	Metabolizable Energy (% of recommendation)[1]				
Quality Trait	94	97	100	SEM	Pr > F
Lightness	54.75	54.52	54.68	0.30	0.8519
Redness	3.51	3.46	3.54	0.10	0.8061
Yellowness	5.64	5.43	5.65	0.11	0.2804
Hue Angle[1]	56.20	55.99	56.56	0.81	0.8814
Chroma[2]	3.00	2.96	3.03	0.03	0.1472
pH_u	5.89	5.88	5.88	0.01	0.7312
Driploss (%)	3.29	3.30	3.28	0.05	0.9755
Cookloss (%)	24.17	23.73	23.65	0.27	0.3527
Myoglobin (mg/g)	0.22	0.18	0.20	0.02	0.3661
Emulsification Capacity (ml oil)	57.87	57.79	57.59	0.08	0.0718
Allo-Kramer Shear (kg F/g)	4.02	4.04	4.06	0.06	0.9021

[a-c] Means within a row with different superscripts differ significantly ($p \leq 0.05$).
[1] ME levels are based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 3.8. Effect of processing age (36, 52 or 56 d) on *P. major* lightness, redness, yellowness, hue, chroma, drip and cooking losses, emulsification capacity, myoglobin content and Allo-Kramer shear values in Cobb x Avian 48 broilers.

	Age (d)						
	36		52		56		
Quality Trait	Mean	SEM	Mean	SEM	Mean	SEM	Pr > F
Lightness	53.15^{b}	0.26	55.16^{a}	0.27	55.64^{a}	0.27	<.0001
Redness	3.75^{a}	0.08	3.49^{b}	0.08	3.27^{b}	0.08	0.0001
Yellowness	4.45^{b}	0.11	6.14^{a}	0.11	6.12^{a}	0.11	<.0001
Hue Angle[1]	48.96^{b}	0.78	59.02^{a}	0.79	60.77^{a}	0.78	<.0001
Chroma[2]	2.85^{b}	0.03	3.10^{a}	0.03	3.05^{a}	0.03	<.0001
pH_u	5.95^{a}	0.01	5.86^{b}	0.01	5.84^{b}	0.01	<.0001
Driploss (%)	3.34^{a}	0.05	3.36^{a}	0.05	3.17^{b}	0.05	0.0145
Cookloss (%)	25.61^{a}	0.26	23.71^{b}	0.26	22.24^{c}	0.29	<.0001
Myoglobin (mg/g)	0.21	0.02	0.19	0.02	0.19	0.02	0.6120
Emulsification Capacity (ml oil)	58.16^{b}	0.09	58.47^{a}	0.07	56.62^{c}	0.07	<.0001
Allo-Kramer Shear (kg F/g)	3.80^{b}	0.05	4.11^{a}	0.05	4.22^{a}	0.05	<.0001

[a-c] Means within a row with different superscripts differ significantly ($p \leq 0.05$).
[1] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[2] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 3.9. Effect of sex on *Pectoralis major* lightness, redness, yellowness, hue, chroma, drip and cooking losses, emulsification capacity, myoglobin content and Allo-Kramer shear values in Cobb x Avian 48 broilers.

	Sex				
	Female		Male		
Quality Trait	Mean	SEM	Mean	SEM	Pr > F
Lightness	55.26[a]	0.24	54.05[b]	0.25	0.0031
Redness	3.52	0.08	3.49	0.08	0.8387
Yellowness	5.78[a]	0.09	5.37[b]	0.09	0.0013
Hue Angle[1]	56.89	0.65	55.60	0.67	0.1838
Chroma[2]	3.03[a]	0.02	2.97[b]	0.02	0.0415
pH_u	5.87	0.01	5.90	0.01	0.0971
Driploss (%)	3.50[a]	0.04	3.08[b]	0.04	<.0001
Cookloss (%)	23.90	0.22	23.81	0.22	0.7731
Myoglobin (mg/g)	0.20	0.01	0.20	0.02	0.9283
Emulsification Capacity (ml oil)	57.70	0.07	57.79	0.07	0.3608
Allo-Kramer Shear (kg F/g)	4.06	0.05	4.03	0.05	0.6465

[a-c] Means within a row with different superscripts differ significantly ($p \leq 0.05$).
[1] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[2] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 3.10. Pearson's correlation coefficents among quality parameters of *P. major* muscle from Cobb x Avian 48 broilers processed at 36, 52 and 56 d

Quality Trait	pH_u[1]	L*[2]	a*[3]	b*[4]	Corn Intake	Hue Angle[5]	Chroma[6]	Driploss %	Cookloss %	A-K Shear[7]	Mb[8]
pH_{24}	1										
n	381										
L*	-0.58**	1									
n	367	378									
a*	0.02	-0.15**	1								
n	362	365	373								
b*	-0.31**	0.30**	0.02	1							
n	367	367	363	378							
Corn Intake	-0.37**	0.24**	-0.19**	0.50**	1						
n	308	378	373	378	392						
Hue Angle	-0.25**	0.36**	-0.52**	0.75**	0.51**	1					
n	377	377	372	378	388	388					
Chroma	-0.29**	0.24**	0.45**	0.88**	0.34**	0.41**	1				
n	377	377	372	378	388	388	388				
Driploss (%)	-0.35**	0.40**	0.06	0.03	-0.15**	-0.04	0.07	1			
n	359	356	352	355	369	365	365	369			
Cookloss (%)	-0.01	0.09	0.18**	-0.07	-0.37**	-0.15**	0.04	0.26**	1		
n	343	342	337	342	354	351	351	334	354		
Allo-Kramer Shear (kg/g)	-0.47**	0.27**	-0.03	0.13*	0.28**	0.13*	0.11*	0.18**	0.20**	1	
n	350	350	345	348	360	358	358	342	334	360	
Myoglobin (mg/g)	0.23**	-0.21	0.05	-0.19**	-0.09	-0.22**	-0.14*	-0.01	0.12	-0.20**	1
n	250	246	247	250	316	255	255	255	228	240	257

* Significant correlations to $p \leq 0.05$
** Significant correlations to $p \leq 0.01$.
[1] pH_{24} = pH 24 h postmortem,
[2] L* = lightness;
[3] a* = redness;
[4] b*=yellowness;
[5] Hue Angle= $\tan^{-1}(b^*/a^*)$
[6] Chroma = $(a^{*2}+b^{*2})^{1/2}$;
[7] A-K Shear = Allo-Kramer shear, kg /g of meat
[8] Mb= myoglobin, mg Myoglobin /g meat.

3.5 FIGURES

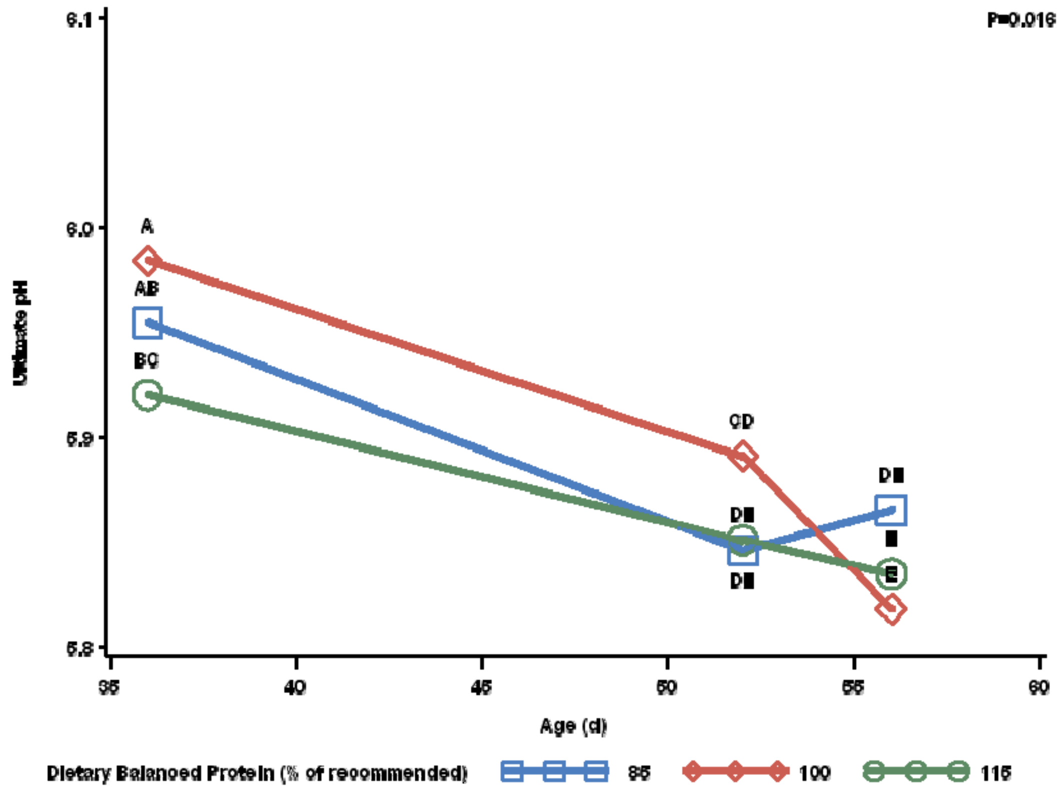

Figure 3.1. Interaction between age at processing (36, 52 and 56 d) and dietary balanced protein (DBP) levels on *Pectoralis major* ultimate pH of Cobb x Avian broilers. 100% DBP is based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio. Points with common letters do not differ significantly (P=0.016).

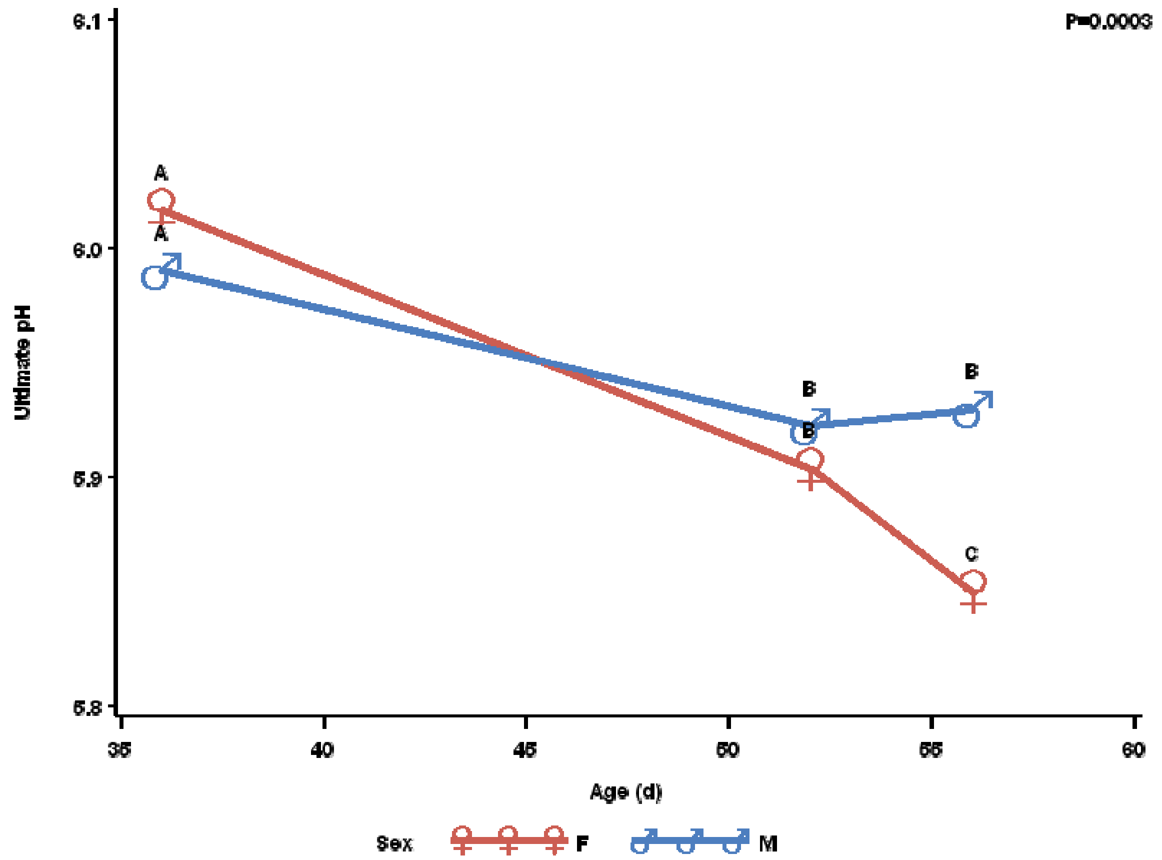

Figure 3.2. Interaction between age at processing (36, 52 and 56 d) and sex on Pectoralis major ultimate pH in Cobb x Avian broilers. Points with common letters do not differ significantly (P=0.0003).

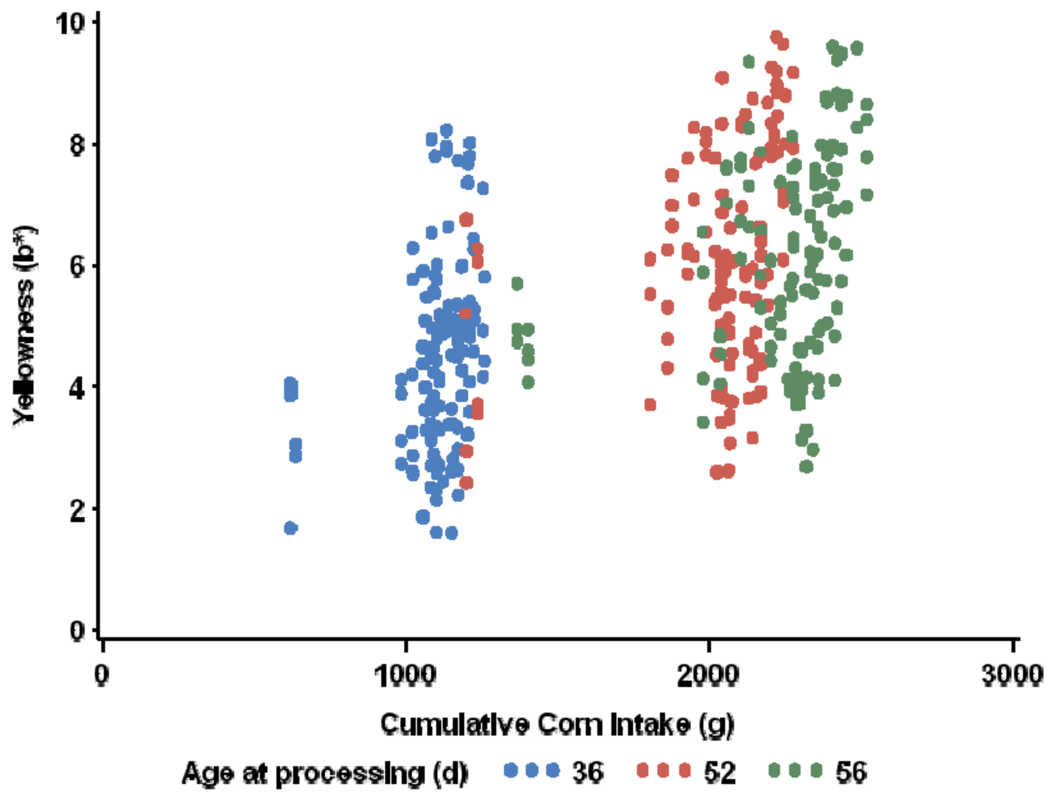

Figure 3.3. Effect of cumulative corn intake on yellowness values of *Pectoralis major* muscle of Cobb-Avian 48 broilers processed at 36, 52, and 56 d.

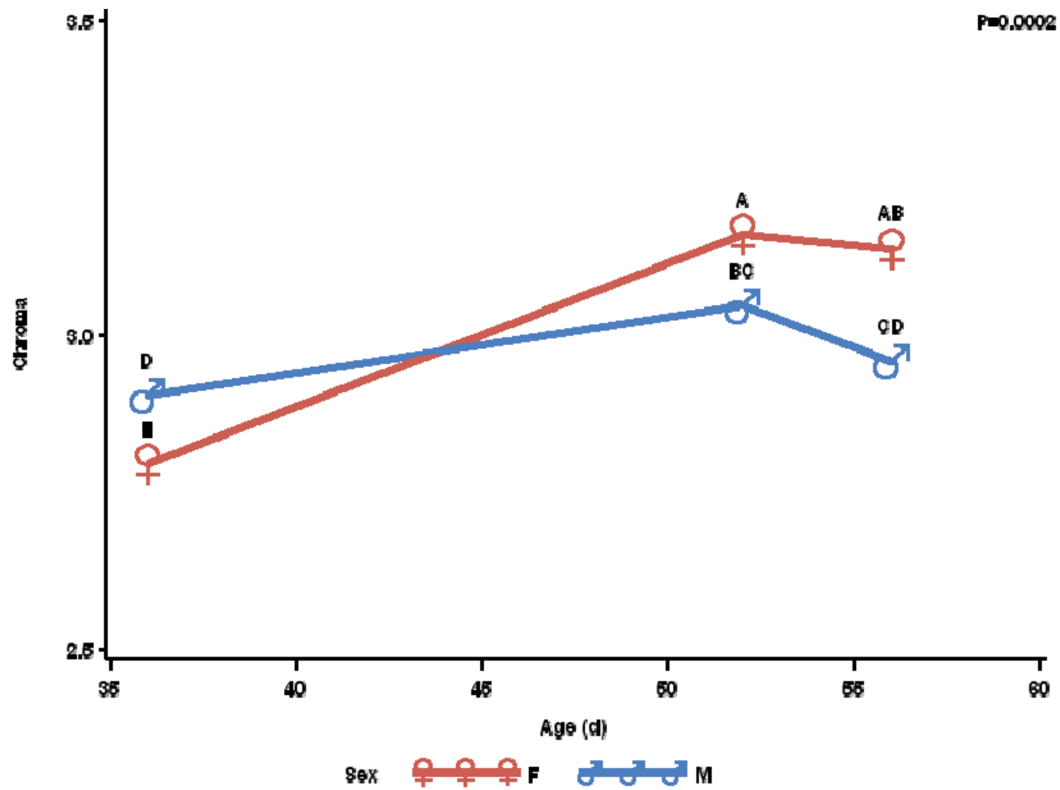

Figure 3.4 Interaction between age at processing (36, 52 and 56 d) and sex on *Pectoralis major* chroma of Cobb x Avian broilers. Chroma refers to the brightness /colorfulness of an object (Schanda, 2007). Points with common letters do not differ significantly (P=0.0002).

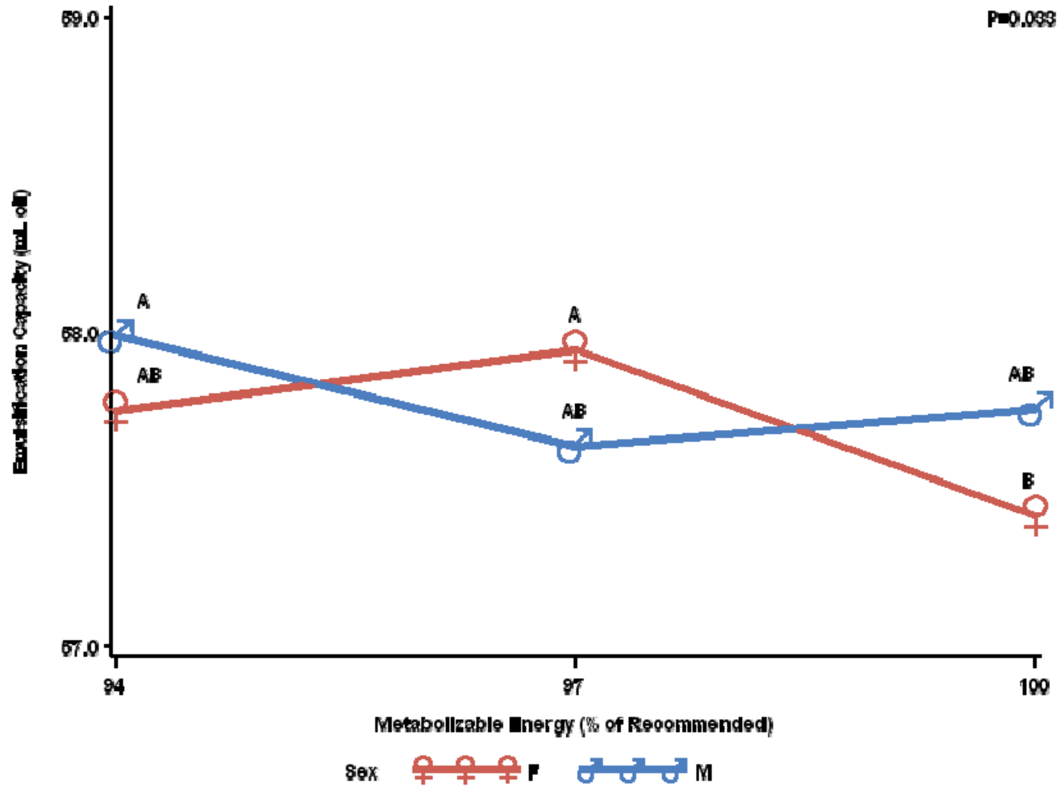

Figure 3.5. Interaction of sex and Metabolizable Energy (ME) level on emulsification capacity (ml oil) in Cobb x Avian 48 broilers processed at 36, 52 and 56 d. 100% metabolizable energy is based on Cobb-Vantress' 2004 commercial recommendations for maximizing growth and feed conversion ratio. Points with common letters do not differ significantly (P=0.0333)

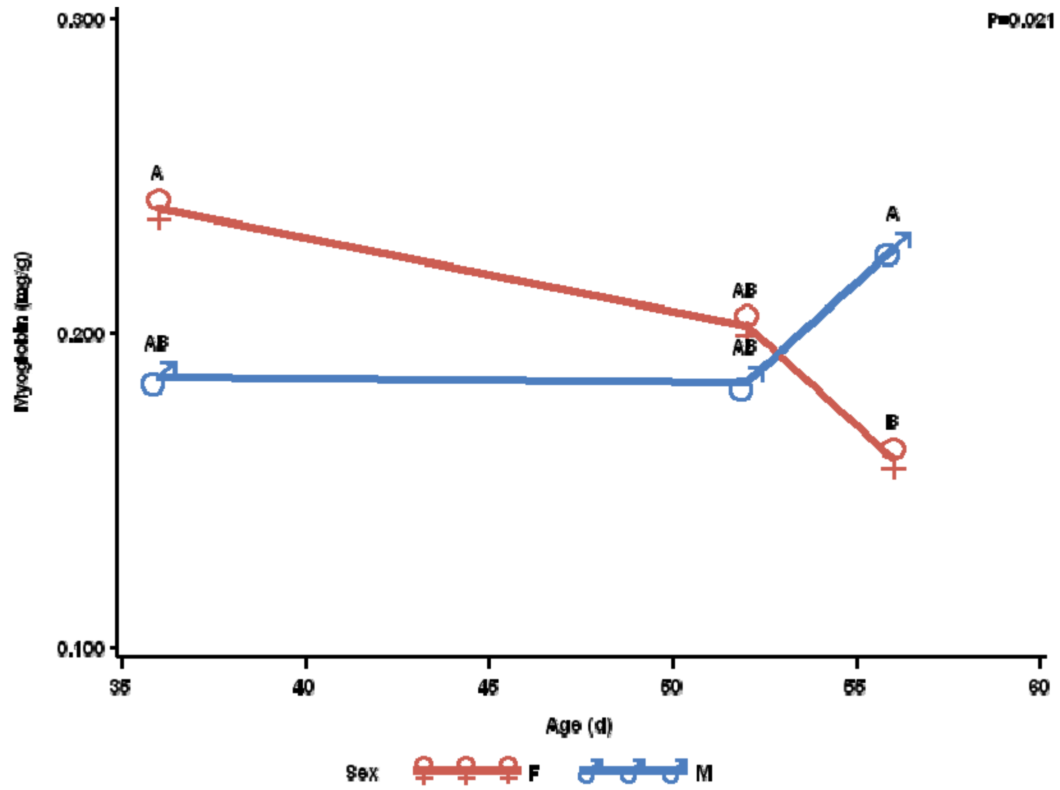

Figure 3.6 Interaction of sex and age on myoglobin content of *Pectoralis major* of Cobb x Avian 48 broilers processed at 36, 52 and 56 d. Points with common letters do not differ significantly (P=0.021)

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ANTE MORTEM HOLDING TEMPERATURE, PERIMORTEM STRUGGLING, SEX AND AGE EFFECTS ON BROILER *PECTORALIS MAJOR* QUALITY

ABSTRACT

Ante mortem holding temperature and *peri mortem* struggling can affect broiler *Pectoralis major* (***P. major***) meat quality. An experiment was performed to investigate the influence of temperature during a 10 h feed withdrawal and *ante mortem* handling on *P. major* functional properties and color. At 28, 35, 42, 49 and 56 d broilers were crated and held in one of three target temperature environments: HEAT (30°C), THERMONEUTRAL (21°C) or COOL (7°C). Following feed withdrawal birds were transported to the processing facility where either a LONG (120 s) or SHORT (<10 s) shackling treatment was imposed. Sex was determined at processing. CIE L* a* b* color was measured at deboning (4.50 to 8.25 h *post mortem*) and at 24 h *post mortem*. Ultimate pH (**pH_u**) was measured at 24 h *post mortem*. Drip loss, cook loss and Allo-Kramer shear force were determined at 72 h *post mortem*. pH_u was highest in the COOL treatment with no difference between HEAT and THERMONEUTRAL treatments (5.97 vs 5.87 and 5.90, respectively; P=0.0004). Differences in pH_u correlated drip loss; drip loss was lowest in the COOL treatment with no difference between the HEAT and THERMONEUTRAL treatments (2.06 vs 2.24 and 2.19%, respectively; $P = 0.007$). *P. major* from HEAT treated broilers had higher Allo-Kramer shear (A-K shear) values than both the COOL and THERMONEUTRAL treatments (4.64 kg F/g vs 4.20 and 4.21 kgF/g, respectively; $P = 0.023$).

With the exception of 49 d, broilers subjected to the LONG shackling treatment had higher redness values than SHORT shackled broilers; by 24 h *post mortem* only the 28 d broilers subjected to the LONG duration had higher redness values. Higher hue angles at deboning and 24 h *post mortem* were observed in the SHORT treatment relative to the LONG treatment (65.58 vs 57.46 and 68.48 vs 63.31, respectively; $P < 0.0001$), indicating that *P. major* from LONG shackled broilers appeared more red overall. Sex significantly affected *P. major* functional properties. pH_u was lower in female broilers than males (5.87 vs 5.96, respectively; $P < 0.0001$); drip loss was subsequently higher in females compared to males (2.34 vs 1.99%, respectively; $P < 0.0001$) Although temperature and handling prior to slaughter affected *P. major* color and texture traits, the differences may not be large enough to have commercial significance. Differences in *P.*

major quality attributable to sex of the bird may have greater commercial significance than previously suspected and could be utilized to improve the efficiency of the broiler supply chain.

KEYWORDS: broiler, meat quality, heat stress, cold stress, shackling

2.8 INTRODUCTION

Variations in breast meat quality are the result of differences in muscle glycogen reserves at slaughter and the rate and extent of pH drop *post mortem* (Owens and Sams, 2000). Feed withdrawal, transport and lairage are stressful events for broilers that occur during the 12 to 14 h period prior to slaughter. High environmental temperature (> 30°C) during this period has been reported to result in a higher incidence of the PSE-like condition (Holm and Fletcher, 1997; Bianchi *et al.*, 2004; 2007; Wang *et al.*, 2009) while cooler temeperatures (4 to 7°C) may result in improved meat quality (Froning *et al.*, 1978; Babji *et al.*, 1982; Dadgar *et al.*, 2008). The PSE-like condition in poultry is the result of an increased rate and extent of glycolysis and lactic acid build-up which results in lower *post mortem* pH; in conjunction with high carcass temperatures accelerated protein denaturation occurs (Offer, 1991; Barbut, 1993; 1997b; 1998; Van Laack *et al.*, 2000). The biochemical mechanisms that cause increased glycolysis *post mortem* are poorly understood (Olivo *et al.*, 2001); however, recent research has indicated that the increased formation of reactive oxygen species (**ROS**) associated with oxidative stress may play a significant role in the acceleration of glycolysis *post mortem* (Betti *et al.*, 2009; Wang *et al.*, 2009). ROS have been implicated in the regulation of glycolysis by adenosine monophosphate protein kinase (**AMPK**) (Betti *et al.*, 2009). AMPK increases cellular energy supplies by activating ATP-generation pathways and decreasing energy demand by deactivating ATP-utilizing pathways (Proszkowiec-Weglarz *et al.*, 2006; Richter and Ruderman, 2009). Broiler or turkey flocks exposed to heat stress conditions prior to slaughter tend to exhibit a higher incidence of the PSE-like condition as evidenced by lower ultimate pH, lighter meat color and poor water holding capacity (**WHC**) (Lee et al., 1976; McCurdy et al., 1996; Holm and Fletcher, 1997; Bianchi et al., 2004; 2007; Wang et al., 2009). Simpson and Goodwin (1975) reported seasonal variation in broiler *Pectoralis major* (***P. major***) tenderness. Heat stress has also been associated with reduced meat tenderness (Lee et al., 1976; Froning et al., 1978; Holm and Fletcher, 1997; Petracci et al., 2001). However, Debut et al. (2003) did not observe any effects of short-term (2 h) heat stress on *P. major* quality.

Broilers or turkeys exposed to cool holding conditions (4-7°C) prior to slaughter tend to exhibit higher ultimate pH and darker meat color (Froning *et al.*, 1978; Babji *et al.*, 1982; Dadgar *et al.*, 2008). The effects of environmental temperature on meat tenderness have been variable depending on temperature and duration of exposure (Wood and Richards, 1975; Froning *et al.*, 1978; Holm and Fletcher, 1997).

Struggling after broilers are placed on a shackle line depletes muscle glycogen stores (Kannan *et al.*, 1997); the amount of muscle glycogen present at death has been reported to be negatively correlated with ultimate pH (**pH_u**). Free struggle during shackling has been shown to lower ultimate pH (Froning *et al.*, 1978; Papinaho *et al.*, 1995), and increase redness and shear values compared to control birds (Froning *et al.*, 1978; Ngoka *et al.*, 1982). Betti *et al*. (2009) suggested that differences in meat tenderness are related to lower ultimate pH and subsequent effects of low pH on moisture loss.

The effect of bird age at slaughter has not been well established for modern birds at current commercial slaughter ages. Froning *et al.* (1968) observed a darkening of turkey meat as processing age increased from 14 to 28 wk, whereas Ngoka *et al.* (1982) did not observe any age related differences between 16 and 20 wk. Smith *et al.* (2002) observed no bird age effects on broiler *P. major* color between 42 and 52 d of age.

Meat tenderness tends to decrease as birds age (Wells and Dawson, 1966; Iqbal *et al.*, 1999; Poole *et al.*, 1999a), likely due to increases in cross-links between collagen molecules (Bailey, 1985). Upon heating, collagen structure transforms from a crystalline structure to an amorphous structure that increases tension in the muscle (Lepetit, 2008). Increased tension forces moisture from the muscle (Kurth, 1993; Lepetit, 2008), thereby reducing tenderness (Kurth, 1993; Betti *et al.*, 2009).

There are few published reports regarding sex-related differences of broiler *P. major* quality. Fanatico *et al* (2005) reported higher yellowness values and lower shear values in females.

Measurements of meat color, specifically L* or lightness values, have been suggested as a tool for identifying meat with poor functional properties (Barbut, 1993; 1998; Fraqueza *et al.*, 2006). When meat samples have been classified into color groups (e.g. light, normal and dark) strong correlations between lightness, pH and subsequent WHC have been observed (Barbut, 1993; Qiao *et al.*, 2001; Woelfel *et al.*, 2002).

The current experiment was designed to measure the effects of environmental temperature during the pre-processing feed withdrawal period and handling prior to

slaughter on *P. major* color and functional properties. The effects of bird age and sex and their interaction with temperature and handling on these properties were also determined.

2.9 MATERIALS AND METHODS

2.9.1 Stocks and Housing

The Animal Care and Use Committee – Livestock of the University of Alberta approved all protocols. Animal care in this experiment met the guidelines of the Canadian Council of Animal Care (1993). A total of 528 Ross 308 mixed-sex broilers obtained from a local hatchery were raised in 8 floor pens on standard University of Alberta rations. Sixty-six birds were placed per pen; stocking density at the beginning of the experiment was 9.57 birds/m^2 decreasing to 2.32 birds/m^2 as birds were removed for sampling. All birds were tagged with wing bands[1] at 21 d.

2.9.2 Holding Temperature

At each of 5 ages (28, 35, 42, 49, and 56 d), 96 broilers were subjected to a 10 h feed withdrawal period at 1 of 3 target holding temperatures: 30°C (**HEAT**), 21°C (**THERMONEUTRAL**) or 7°C (**COOL**). At 28 and 35 d of age 8 birds were placed per crate; at 42, 49 and 56 d of age 4 birds were placed per crate. Actual temperature inside individual crates and the environment chamber was recorded using Microlog[2] temperature loggers. Outdoor ambient temperature during the feed withdrawal period was obtained from Environment Canada[3].

2.9.3 Shackling Treatments

After the feed withdrawal period, the crates were transported to the processing plant where birds were shackled for 120 s prior to stunning (**LONG**), or shackled < 10 s (**SHORT**) prior to stunning. Flapping was induced in the LONG treatment by lifting each bird from an inverted position on the shackles to a straightened position (whereby the head of the bird was over the legs) after which the bird was subsequently returned to an inverted position; this process was repeated several times over the 120 s shackling period. Birds in the SHORT treatment were handled in a manner that minimized flapping.

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[1] National Band and Tag, Newport, CT 41072-0430
[2] Fourier Systems, Fairfield, CT 06824
[3] Environment Canada, Gatineau, PQ K1A 0H3 www.ec.gc.ca

2.9.4 Processing and Sample Preparation

Carcasses were scalded for 45 s at 63°C and mechanically defeathered and eviscerated. Sex was determined during evisceration. Carcasses were deboned after chilling to an internal temperature of 4°C as required by HACCP protocols. The average time required to chill carcasses to 4°C after slaughter increased with age from 3:47 h at 28 d to 7:52 h at 56 d, likely due to increasing size of the carcass with age. Eviscerated carcass and *Pectoralis major* (*P. major)* weights were recorded at deboning.

2.9.4.1 ***Color***

A Minolta CR-400[4] colorimeter using illuminant D65 as the light source was used to assess the color (CIE L* a* b*) of *P. major* muscle. L* refers to lightness, a* refers to redness and b* refers to yellowness. Color was measured at deboning and at 24 h *post mortem* on the cranial, medial surface (bone side) of individual *P. major* fillets in an area free from obvious color defects (bruises, blood spots, or surface discolorations). The mean time between slaughter and the deboning color measurement increased with age from 4:33 h at 28 d to 8:18 h at 56 d. Meat color values have been reported to change rapidly between 0 to 12 h *post mortem* (Barbut, 1998; Petracci and Fletcher, 2002). Three readings per fillet were taken and the average reading was recorded. Hue angle, ($\tan^{-1}(b/a)$), measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006). As hue angle increases, visually perceived redness decreases (Little, 1975; Brewer *et al.*, 2006). Chroma ($(a^2+b^2)^{1/2}$) refers to the brightness/colorfulness of an object (Fairchild, 2004).

2.9.4.2 ***Ultimate pH***

Ultimate pH was measured via direct insertion of a Hanna Instruments[5] electrode to each fillet at 24 h *post mortem*. An incision 0.5 to 1 cm deep was made to allow insertion of the electrode. Equipment malfunctions at 28 and 35 d resulted in loss of data for those ages.

2.9.4.3 ***Water Holding Capacity***

A core sample (25 x 50 mm) was excised from the cranial end of the *P. major* fillet and weighed to assess WHC. The core sample was suspended on cheese cloth for 48 h in a sealed plastic container and reweighed at 72 h *post mortem*. Drip loss is expressed as a percentage of the initial weight of the core sample. Cooking loss was evaluated at 72 h *post mortem* by cooking at 163 C in a convection oven until an internal

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[4] Konica Minolta Sensing Americas, Inc, Ramsey, NJ 07446
[5] Hanna® Instruments, Woonsocket, RI, 02895 USA

temperature of 80 C was reached[6]. The samples were cooled to room temperature and reweighed to determine cooking loss (expressed as a percentage of initial weight of the core sample).

2.9.4.4 *Allo-Kramer Shear Determination*

Following cooking loss determination, A-K shear values were measured at 72 h *post mortem* using an Instron Universal Testing Machine[7] equipped with an Allo-Kramer cell. The same sample used for cooking loss determination was placed with the blades at a right angle to the muscle fibers using a 200-kg load cell and cross head speed of 100 mm/min. Shear values are reported as kilograms of force per gram of sample.

2.9.4.5 *Protein Solubility*

Total and sarcoplasmic protein solubility were measured according to the method described by Van Laack *et al.* (2000). Following protein extraction, samples were stored at -20 C for 6 mo prior to analysis. Sarcoplasmic protein samples from d 49 were lost.

2.9.5 Statistical Analysis

Analysis of variance was performed by the Mixed Model procedure of SAS® (SAS Institute Inc., 2008) for a 4 level split-plot design with crate as the experimental unit. The following model was used:

$Y_{ijklm} = \mu + T_i + C_i + Sh_j + ShT_{ij} + C_{(ij)} + S_k + ST_{ik} + SSh_{jk} + A_l + AT_{il} + ASh_{jl} + AS_{kl} + \varepsilon_{ijklm}$

Where Y_{ijklm}= variable measured for the mth bird, μ = overall mean, T_i = effect of the ith temperature; C_i = effect of the ith crate within temperature, temperature error component; Sh_j = effect of the jth shackling treatment; ShT_{ij} =interaction between shackling and temperature; $C_{(ij)}$ = shackling error component; S_k = effect of the kth sex; ST_{ik} = interaction between sex and temperature; SSh_{jk} = interaction between sex and shackling; A_l = effect of lth age; AT_{il} = interaction between age and temperature; ASh_{jl} = interaction between age and shackling; AS_{kl} = interaction between age and sex; ε_{ijklm} = split-plot error component.

The mean time between slaughter and the deboning color measurement increased with age. Since meat color values change rapidly between 0 to 12 h *post mortem* (Barbut, 1998; Petracci and Fletcher, 2002), the time between slaughter and the deboning color measurement was used as a covariate in the statistical analysis to account for time of deboning differences in color. The model presented above was modified to:

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[6] Model Digi-Sense RK-92000 Benchtop 115V, Cole Parmer Instrument Co, Montreal, QC H4P 2R9

[7] Model 4411, Instron Corp., Canton, MA

$Y_{ijklm} = \mu + T_i + C_i + Sh_j + ShT_{ij} + C_{(ij)} + S_k + ST_{ik} + SSh_{jk} + A_l + AT_{il} + ASh_{jl} + AS_{kl} + \beta(Time_{ijklm} - Time_a) + \varepsilon_{ijklm}$

Where $\beta(Time_{ijklm} - Time_a)$ = a covariate coefficient which is multiplied by the difference between the individual time between slaughter and color measurement ($Time_{ijklm}$) and the average time between slaughter and color measurement ($Time_a$) to account for the covariate effect.

Means were separated using least square means and PDIFF was assessed for significance ($P \leq 0.05$). The SEM for the group with the least number of birds was reported. Pearson correlation coefficients were calculated using the Corr procedure of SAS® (SAS Institute Inc., 2008).

2.10 RESULTS AND DISCUSSION

2.10.1 Actual Temperatures

Over the course of the experiment, average temperature in the COOL treatment was 9.8°C. Average temperature in the THERMONEUTRAL treatment was 23.2°C. The average temperature in the HEAT treatment was 28.8°C (Figure 0.1). Average outdoor temperatures during the feed withdrawal period ranged between 3.84 C and 14.22 C over the 5 ages (Figure 0.1).

2.10.2 Holding Temperature

Meat color is largely influenced by heme pigment concentration (Fleming *et al.*, 1991; Cornforth, 1994; Froning, 1995; Warriss, 2000; Barbut, 2002) and the rate and extent of *post mortem* glycolysis (McKee and Sams, 1998; Alvarado and Sams, 2002; Sams and Alvarado, 2004). Holding temperature during feed withdrawal affected lightness and yellowness at deboning; meat from the HEAT and THERMONEUTRAL groups was lighter than the COOL group (53.77 and 54.32 vs 53.21, respectively P=0.0004; Table 0.1). Meat was less yellow at deboning in the COOL group compared to the THERMONEUTRAL group, while the HEAT group was not different from either (5.38, 5.66 and 5.55, respectively, P=0.0439; Table 0.1). pH_u was highest in the COOL treatment, with no differences observed between the HEAT and THERMONEUTRAL groups (5.97 vs 5.87 and 5.90, respectively, P=0.0004;). Drip loss was lowest in the COOL treatment, with no differences observed between the HEAT and THERMONEUTRAL treatments (2.06 vs 2.24 and 2.19, respectively, P=0.0071; Table 0.1). Maintenance of a constant internal body temperature in homeotherms has a high energy cost (Silva, 2006). Mammals or birds exposed to cool temperatures generate heat

by one of two processes – shivering or non-shivering thermogenesis (Hocquette *et al.*, 1998). Shivering is an involuntary contraction of myofibrils that utilizes ATP and increases the rate of energy substrate oxidation (Hocquette *et al.*, 1998). Reduced availability of energy substrates at slaughter after cold exposure likely limits lactic acid production, resulting in higher pH_u while limiting drip losses. A significant negative correlation between pH_u and drip loss was observed (r=-0.47,Table 0.2) which supports this conclusion. This indicates that functional properties of broiler meat can be improved by exposing broilers to cool temperatures (4-7°C) during the 12 to 14 h period prior to slaughter; however, high pH has also been associated with shorter shelf life which may negate these effects (Allen *et al.*, 1997).

Allo-Kramer shear values observed in the HEAT treatment were higher than both COOL and THERMONEUTRAL samples, which were not different from each other (4.64 vs 4.21 and 4.20 kgF/g, respectively, P=0.023; Table 0.1). Similar effects on *P. major* tenderness after exposure to high temperatures have been reported (Lee *et al.*, 1976; Froning *et al.*, 1978; Babji *et al.*, 1982; Petracci *et al.*, 2001). Betti *et al.* (2009) reported higher A-K shear when pH_u was below 5.72; tenderness differences were attributed to decreases in WHC associated with low pH_u. Cook loss and A-K shear values in the present experiment were positively correlated (r=0.44; Table 0.2) which supports the conclusion that moisture losses during cooking negatively affect meat tenderness. The present experiment observed a similar trend in the HEAT treatment (low pH_u and high drip loss in conjunction with high A-K shear values). Regardless, A-K shear values under 6.0 kg F/g have been previously correlated with 'very tender' to 'moderately tender' sensory panel scores (Lyon and Lyon, 1990). As the A-K shear values in the present study were all below 6.0 kg F/g, the difference due to heat stress should not affect consumer perception of meat tenderness.

It is surprising that limited effects of heat stress were observed in the present study. It is possible that 30°C temperatures were not high enough to elicit increased ROS formation similar to that observed by Wang *et al.* (2009) in birds exposed to 40°C for 1 to 5 h.

2.10.3 Shackling Duration

In the current study, lightness values at 24 h *post mortem* were higher in the SHORT treatment compared to the LONG treatment (56.51 vs 55.91, respectively; Table 0.3). Overall, the LONG duration resulted in higher redness values at deboning and 24 h *post mortem* than the SHORT duration (3.31 vs 2.62 and 2.95 vs 2.49, respectively; Table

0.3). However, an interaction between shackling duration and age was observed for redness values at deboning and 24 h *post mortem*. With the exception of 49 d, broilers subjected to the LONG shackling treatment had higher *P. major* redness values at deboning (Table 0.3); by 24 h *post mortem* only the 28 d broilers subjected to the LONG duration had higher redness values. Previous studies have observed meat color changes in birds that struggled freely on the shackling line (Froning *et al.*, 1978; Kannan *et al.*, 1997). In a study designed to simulate acute *ante mortem* stress, epinephrine injections resulted in darker meat due to higher pH_u and increased haemoglobin concentrations (Walker and Fletcher, 1993). Fletcher (2005) observed that flapping resulted in more red *P. minor* muscles, but did not observe consistent results with *P. major* fillets. Kannan *et al.* (1997) theorized that the color differences affected by shackling time likely would not have commercial significance. The present study supports a similar conclusion, since the differences in absolute redness values in the present study, while statistically significant, would not likely be perceived by consumers. Furthermore, an increase in hue angles in both the LONG and SHORT groups between deboning and 24 h *post mortem* (57.46 to 63.31 and 65.58 to 68.48, respectively; Table 0.3) indicate a decrease in perceived redness over time. The increase in redness values in the LONG treatment in the present study may have been due to an increase in total heme pigments, which can be elevated by stress prior to slaughter (Walker and Fletcher, 1993).

At 42 d, broilers subjected to the LONG shackling treatment had lower pH_u than the SHORT duration, at 49 and 56 d there were no differences in pH_u between shackling treatments (Table 0.4).

Surprisingly, SHORT shackling resulted in higher A-K shear values than the LONG duration, (4.61 vs 4.08 kg F/g, respectively; Table 0.3). Kannan *et al*. (1997) subjected broilers to 0, 2 or 4 minutes of shackling and observed that shackling for 2 or 4 min resulted in lower A-K shear values than 0 min; however, the differences were not significant. In contrast, Froning *et al.* (1978) observed higher shear values in turkey *P. major* from birds that freely struggled compared to anesthetized birds. The lower A-K shear values observed in the LONG treatment could be the result of increased proteolytic activity by calpain. Vigorous flapping immediately prior to slaughter may have increased the concentration of calcium ions in the sarcoplasm, activating the calpain proteolytic system. An interaction between shackling duration and age indicates that shackling has an inconsistent effect on meat tenderness as bird age increases (Table 0.4). At 28, 49 and 56 d there were no differences between LONG and SHORT shackling treatments; at 35

and 42 the SHORT shackled birds had higher A-K shear values compared to LONG shackled birds (Table 0.4). At all ages, A-K shear values in the LONG and SHORT groups were under the threshold that consumers would perceive as tender (Lyon and Lyon, 1990).

2.10.4 Sex of Bird

Although most published reports do not describe any sex-related differences in broiler *P. major* functional properties or color, some interesting trends emerged from the present experiment. *P. major* samples from female broilers in the current study had higher lightness values at deboning and 24 h *post mortem*, had lower pH_u and higher drip loss compared to males (Table 0.5). Interactions were observed between sex and age for cook loss, chroma and lightness values at deboning and 24 h *post mortem*. At 28, 35 and 56 d there were no sex-related differences in cook losses (Figure 0.2). However, at 42 d male samples had higher cook loss than females, while at 49 d females had higher cook loss. Since no differences were attributable to sex at most of the ages studied and neither males nor females were consistently higher at 42 and 49 d, commercial significance is likely limited.

Chroma values at deboning were higher in females at 56 d (Figure 0.3); however, sex related differences were observed at 49 and 56 d for chroma values at 24 h *post mortem* (Figure 0.4). *P. major* lightness values at deboning were higher in females after 35 d (Figure 0.5), while lightness values at 24 h *post mortem* were higher after 42 d (Figure 0.6). Ultimate pH was higher in males compared to females (5.96 vs 5.87, respectively; Table 0.5). Low ultimate pH (<5.8) is associated with reduced WHC as pH approaches muscle isoelectric point of myofibrillar proteins (Barbut, 1998). The lower pH observed in the female broilers was approaching the isoelectric point; this likely contributed to the higher drip losses observed in females relative to males (2.34 vs 1.99%, respectively; Table 0.5). No differences were observed in tenderness or total protein solubility between the sexes. Sarcoplasmic protein solubility was higher in females compared to males (85.5 vs 84.0 mg/g, Table 0.5), indicating that more sarcoplasmic protein denaturation occurred in males. Although drip loss was higher in females, differences in sarcoplasmic protein solubility are not the major contributors to WHC; myofibrillar protein has a greater influence on WHC than sarcoplasmic protein (Van Laack *et al.*, 2000). Sex-related differences in pH_u and subsequent effects on drip loss and meat color may be the result of differences in muscle glycogen content or different regulatory patterns of *post mortem* metabolism.

2.10.5 Age

Bird age affected all variables (Table 0.6). As mentioned earlier, the interaction between shackling duration and age demonstrated that LONG shackling decreased pH_u at 42 d with no differences at later ages. Ultimate pH at 49 d was higher than 56 d; 42 d pH_u was not different from either 49 or 56 d (5.94 vs 5.89 and 5.91). Redness values were affected by an interaction between age and shackling duration; overall redness at deboning and 24 h *post mortem* decreased (Table 0.6). Hue angles also increased with age which indicates that meat appears less red (Table 0.6). Meat lightness increased with age, but as previously discussed, was also affected by the sex of the bird. These results are counter to results reported by Smith *et al.* (2002), who observed no differences in *P. major* color as broilers aged from 42 to 52 d. In contrast to the present study, Fletcher (2002) theorized that breast muscle becomes darker and more red with age due to an increase in myoglobin in muscle. Drip loss and A-K shear values varied with age with no increasing or decreasing trends as bird age increased. The lack of a consistent decrease in A-K shear values with age is surprising, considering that older animals typically have a higher number of collagen cross-links. Increased cross-links are associated with decreased tenderness due to connective tissue shrinkage and subsequent fluid expulsion during cooking (Kurth, 1993; Lepetit, 2008). It is therefore interesting to note that lower cook losses tended to be associated with decreased A-K shear values; for example, at 28 d cook losses of 20.47% were observed in conjunction with one of the lowest A-K shear values (4.09 kg force/g). This is in agreement with Betti *et al.* (2009), who observed decreased tenderness when cook losses were high. The interaction between sex and age on cook loss has been discussed previously; overall, cook loss increased with age between 28 and 49 d, at 56 d cook loss was the same as at 28 d (Table 0.6). Total and sarcoplasmic protein solubility tended to increase with age, possibly the result of an increase in overall protein deposition in *P. major*. The largely inconsistent trends observed due to age indicate that factors other than those measured in the present study may have affected meat quality parameters.

2.10.6 Correlations

Lightness at deboning was strongly correlated to other color measurements; however, the relationship between lightness at deboning and drip loss (0.11, n=439) and cook loss (0.10, n=446) were quite low compared to previous studies (Barbut, 1993; 1996; 1997b; Owens *et al.*, 2000; Woelfel *et al.*, 2002). Meat color, specifically lightness, has been suggested as a screening tool to detect PSE-like meat (Barbut, 1993). Several

studies have reported strong correlations between muscle pH and color when categorizing meat samples into pale, normal or dark categories (Van Laack *et al.*, 2000; Qiao *et al.*, 2001; Qiao *et al.*, 2002; Barbut *et al.*, 2005). This may be problematic, since it has been suggested that the relationship between pH and meat lightness is non-linear (Owens *et al.*, 2000). Studies that categorize meat as pale, normal or dark may not accurately describe the relationships between color, pH and WHC.

2.11 CONCLUSIONS

This study demonstrated that cool temperatures during the feed withdrawal period resulted in higher pH, darker meat, lower drip loss and lower A-K shear values. Increased struggling resulted in lower A-K shear values and higher redness values. Although temperature and handling prior to slaughter affected *P. major* color and tenderness, the differences may not be large enough to have commercial significance. Sex and age of bird may have a greater influence on functional properties of broiler *P. major* than previously reported; further study is required to understand how these differences can be utilized to the benefit the broiler supply chain.

2.12 TABLES

Table 0.1. The effect of holding temperature (Cool 7C, Thermoneutral 21 C or Heat 30 C) during 10 h feed withdrawal period on *Pectoralis major* color at deboning[1] and 24 h *post mortem* and functional properties in Ross 308 broilers at 5 processing ages.

	Holding Temperature				
Quality Trait	Cool	Heat	Thermoneutral	SEM	Pr > F
$Lightness_{debone}$	53.22^b	53.82^a	54.13^a	0.20	0.004
$Lightness_{24}$	55.87	56.34	56.45	0.24	0.225
$Redness_{debone}$	2.97	2.97	2.93	0.08	0.943
$Redness_{24}$	2.79	2.69	2.66	0.09	0.570
$Yellowness_{debone}$	5.38	5.55	5.61	0.09	0.180
$Yellowness_{24}$	6.00	6.15	6.22	0.11	0.318
Hue $Angle_{debone}$ [2]	60.89	61.56	61.96	0.88	0.672
$Chroma_{debone}$ [3]	6.29	6.46	6.47	0.09	0.318
Hue $Angle_{24}$ [2]	64.68	66.43	66.77	0.79	0.126
$Chroma_{24}$ [3]	6.80	6.85	6.95	0.11	0.620
pH_{24}	5.97^a	5.87^b	5.90^b	0.02	0.001
Driploss (%)	2.06^b	2.24^a	2.19^a	0.04	0.010
Cookloss (%)	23.56	24.03	23.60	0.31	0.497
Allo-Kramer Shear (kg Force/g)	4.21^b	4.63^a	4.21^b	0.13	0.033
Protein Solubility (mg/g)					
Total protein	175.00	165.88	170.01	3.25	0.154
Sarcoplasmic protein	85.59	85.80	82.92	1.35	0.185

[a-b] Means within a row without common superscripts differ significantly ($P < 0.05$).
[1] Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 0.2. Pearson correlation coefficients and correlation significance among quality parameters of *P. major* samples from Ross 308 broiler carcasses at 28, 35, 42, 49 and 56 d.

Quality Parameters	pH_u[1]	L^*_{debone}[23]	a^*_{debone}[4]	b^*_{debone}[5]	L^*_{24}[6]	a^*_{24}[7]	b^*_{24}[8]	Driploss %	Cookloss %	*A-K Shear*[9]
pH_u	1									
n	273									
L^*_{debone}	-0.44**	1								
n	268	464								
a^*_{debone}	-0.08	-0.50**	1							
n	273	460	468							
b^*_{debone}	-0.35**	0.61**	-0.14**	1						
n	273	460	465	468						
L^*_{24}	-0.46**	0.74**	-0.40**	0.46**	1					
n	268	455	459	458	465					
a^*_{24}	-0.21**	-0.39*	0.76**	-0.23**	-0.32**	1				
n	272	457	462	461	464	468				
b^*_{24}	-0.39**	0.50**	-0.18**	0.50**	0.70**	0.05	1			
n	268	454	460	458	462	464	465			
Driploss (%)	-0.47**	0.11*	0.22**	0.07	0.24**	0.28**	0.21**	1		
n	266	439	443	442	441	444	440	449		
Cookloss (%)	-0.04	0.10*	-0.02	0.21**	0.08	-0.25**	-0.09	-0.03	1	
n	260	446	450	450	446	449	446	433	456	
Allo-Kramer Shear (kgF/g)	-0.10	0.05	-0.06	0.09	0.15**	-0.12*	0.07	0.13**	0.44**	*1*
n	*254*	*431*	*434*	*433*	*431*	*434*	*431*	*416*	*430*	*440*

[1] pH_u = pH measured at 24 h postmortem, age 42, 49 and 56 d;
[2] Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d
[3] L^*_{debone} = lightness at deboning
[4] a^*_{debone}= redness at deboning
[5] b^*_{debone}=yellowness at deboning
[6] L^*_{24}= lightness at 24 h *post mortem*
[7] a^*_{24}= redness at 24 h *post mortem*
[8] b^*_{24}=yellowness at 24 h *post mortem*
[9] A-K Shear = Allo-Kramer shear, kg Force/g of meat
* Significant orrelations to $p < 0.05$
** Significant correlations to $p < 0.01$.

Table 0.3. The effect of long (120 s) or short (<10 s) shackling duration on *Pectoralis major* color at deboning[1] and 24 h *post mortem* and functional properties in Ross 308 broilers at 5 processing ages.

	Shackling Duration			
Quality Trait	Long	Short	SEM	Pr > F
$Lightness_{debone}$	53.67	53.77	0.16	0.684
$Lightness_{24}$	55.95	56.49	0.20	0.070
$Redness_{debone}$	3.29^{a}	2.62^{b}	0.07	<.0001
$Redness_{24}$	2.94^{a}	2.49^{b}	0.07	<.0001
$Yellowness_{debone}$	5.37^{b}	5.66^{a}	0.07	0.008
$Yellowness_{24}$	5.97^{b}	6.28^{a}	0.09	0.011
Hue $Angle_{debone}$ [2]	57.62^{b}	65.32^{a}	0.71	<.0001
$Chroma_{debone}$ [3]	6.47	6.35	0.07	0.253
Hue $Angle_{24}$ [2]	63.42^{b}	68.50^{a}	0.64	<.0001
$Chroma_{24}$ [3]	6.86	6.88	0.09	0.867
pH_{24}	5.91	5.91	0.01	0.961
Driploss (%)	2.18	2.15	0.03	0.473
Cookloss (%)	23.93	23.52	0.25	0.268
Allo-Kramer Shear (kg Force/g)	4.10^{b}	4.60^{a}	0.10	0.002
Protein Solubility (mg/g)				
Total protein	167.84	172.76	2.57	0.186
Sarcoplasmic protein	84.70	84.83	0.97	0.918

[a-b] Means within a row without common superscripts differ significantly ($P < 0.05$).
[1] Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 0.4. Interaction between long (120 s) and short (<10 s) shackling duration and age at processing (28, 35, 42, 49, 56) on redness at deboning and 24 h *post mortem*, chroma[1] at deboning[2], hue angle[3] at 24 h *post mortem* and Allo-Kramer shear values of Ross 308 *P. major* samples.

Quality Trait	Shackling Duration	Age at Processing (d)					SEM	Pr > F
		28	35	42	49	56		
Redness_{debone}	Long (120 s)	5.03^{a}	3.86^{b}	3.43^{c}	2.69^{d}	1.46^{e}	0.16	0.012
	Short (< 10 s)	3.93^{b}	2.95^{d}	2.68^{d}	2.57^{d}	0.96^{f}	0.16	0.012
Redness_{24}	Long (120 s)	5.10^{a}	4.00^{b}	1.90^{c}	1.78^{cd}	1.89^{cd}	0.15	0.001
	Short (< 10 s)	3.87^{b}	3.65^{b}	1.62^{cd}	1.85^{cd}	1.47^{d}	0.15	0.001
Chroma_{debone}[1]	Long (120 s)	7.11^{a}	6.47^{bcd}	6.62^{bc}	6.20^{cde}	5.95^{de}	0.17	0.015
	Short (< 10 s)	6.64^{bc}	6.41^{bcd}	6.15^{de}	6.71^{ab}	5.83^{e}	0.16	0.015
Hue Angle_{24}[2]	Long (120 s)	48.39^{e}	55.03^{d}	69.35^{b}	71.33^{b}	73.00^{b}	1.44	0.016
	Short (< 10 s)	59.35^{c}	58.41^{cd}	72.95^{b}	73.14^{b}	78.64^{a}	1.43	0.016
pH_u[3]	Long (120 s)			5.87^{c}	5.98^{a}	5.90^{bc}	0.02	0.006
	Short (< 10 s)			5.94^{ab}	5.92^{abc}	5.88^{c}	0.02	0.006
Allo-Kramer Shear (kg Force/g)	Long (120 s)	4.12^{c}	3.68^{d}	4.28^{bc}	4.41^{bc}	4.03^{cd}	0.17	0.001
	Short (< 10 s)	4.07^{cd}	4.28^{bc}	5.50^{a}	4.73^{b}	4.41^{bc}	0.17	0.001

[a-f] Means within a parameterwithout common superscripts differ significantly ($P < 0.05$).
[1]Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 0.5. Effect of sex on color at deboning[1] and 24 h post mortem and functional properties of Pectoralis major in Ross 308 broilers processed at 28, 35, 42, 49 or 56 d.

	Sex			
Quality Trait	Female	Male	SEM	Pr > F
$Lightness_{debone}$	54.55^a	52.89^b	0.17	<.0001
$Lightness_{24}$	57.02^a	55.43^b	0.20	<.0001
$Redness_{debone}$	2.80^b	3.11^a	0.07	0.001
$Redness_{24}$	2.67	2.76	0.07	0.345
$Yellowness_{debone}$	5.69^a	5.34^b	0.07	<.0001
$Yellowness_{24}$	6.49^a	5.76^b	0.09	<.0001
Hue $Angle_{debone}$ [2]	63.58^a	59.36^b	0.73	<.0001
$Chroma_{debone}$ [3]	6.51^a	6.31^b	0.07	0.042
Hue $Angle_{24}$ [2]	67.62^a	64.30^b	0.65	<.0001
$Chroma_{24}$ [3]	7.16^a	6.58^b	0.09	<.0001
pH_{24}	5.87^b	5.96^a	0.01	<.0001
Driploss (%)	2.34^a	1.99^b	0.03	<.0001
Cookloss (%)	23.71	23.74	0.23	0.923
Allo-Kramer Shear (kg Force/g)	4.36	4.34	0.08	0.875
Protein Solubility (mg/g)				
Total protein	171.66	168.94	2.60	0.455
Sarcoplasmic protein	85.55^a	83.99^b	0.81	0.033

[a-b] Means within a row without common superscripts differ significantly ($P < 0.05$).
[1]Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

Table 0.6. Effect of age on *Pectoralis major* color at deboning[1] and 24 h *post mortem* and functional properties of Ross 308 broilers

	Age at Processing (d)						
Quality Trait	28	35	42	49	56	SEM	Pr > F
Lightness$_{debone}$	51.39[d]	52.45[c]	52.17[c]	54.87[b]	57.72[a]	0.36	<.0001
Lightness$_{24}$	54.55[d]	55.39[c]	55.14[cd]	56.59[b]	59.44[a]	0.31	<.0001
Redness$_{debone}$	4.48[a]	3.41[b]	3.05[c]	2.63[d]	1.21[e]	0.15	<.0001
Redness$_{24}$	4.49[a]	3.82[b]	1.76[c]	1.81[c]	1.68[c]	0.11	<.0001
Yellowness$_{debone}$	5.13[c]	5.41[bc]	5.51[b]	5.85[a]	5.66[ab]	0.15	<.0001
Yellowness$_{24}$	6.27[b]	5.78[c]	5.11[d]	6.27[b]	7.19[a]	0.14	<.0001
Hue Angle$_{debone}$ [2]	48.60[d]	58.33[c]	61.39[c]	65.40[b]	73.63[a]	1.57	<.0001
Chroma$_{debone}$ [3]	6.87[a]	6.44[b]	6.39[b]	6.46[b]	5.89[c]	0.15	<.0001
Hue Angle$_{24}$ [2]	53.87[d]	56.72[c]	71.15[b]	72.24[b]	75.82[a]	1.05	<.0001
Chroma$_{24}$ [3]	7.83[a]	7.00[b]	5.45[d]	6.60[c]	7.46[a]	0.14	<.0001
pH$_{24}$			5.91[b]	5.95[a]	5.89[b]	0.01	0.018
Driploss (%)	2.46[a]	2.19[b]	2.36[a]	1.69[c]	2.12[b]	0.06	<.0001
Cookloss (%)	20.47[d]	22.48[c]	26.93[b]	28.20[a]	20.55[d]	0.36	<.0001
Allo-Kramer Shear (kg Force/g)	4.09[c]	3.98[c]	4.89[a]	4.57[b]	4.22[c]	0.13	<.0001
Protein Solubility (mg/g)							
Total protein	140.12[d]	156.06[c]	186.17[ab]	191.05[a]	178.10[b]	7.39	<.0001
Sarcoplasmic protein	80.95[c]	77.52[d]	87.34[b]		93.27[a]	1.12	<.0001

[a-d] Means within a row without common superscripts differ significantly ($P < 0.05$).
[1]Carcasses were deboned after chilling to an internal temperature of 4 C as required by HACCP protocols. The mean time between slaughter and chilling to 4 C increased with age from 3:47 h at 28 d to 7:52 h at 56 d.
[2] Hue Angle $\tan^{-1}(b/a)$. Hue angle, as calculated from a* and b* values, measures the degree of departure from the true red axis of the CIE color space (Brewer *et al.*, 2006)
[3] Chroma = $(a^2+b^2)^{1/2}$ Chroma refers to the brightness/colorfulness of an object (Schanda, 2007).

2.13 FIGURES

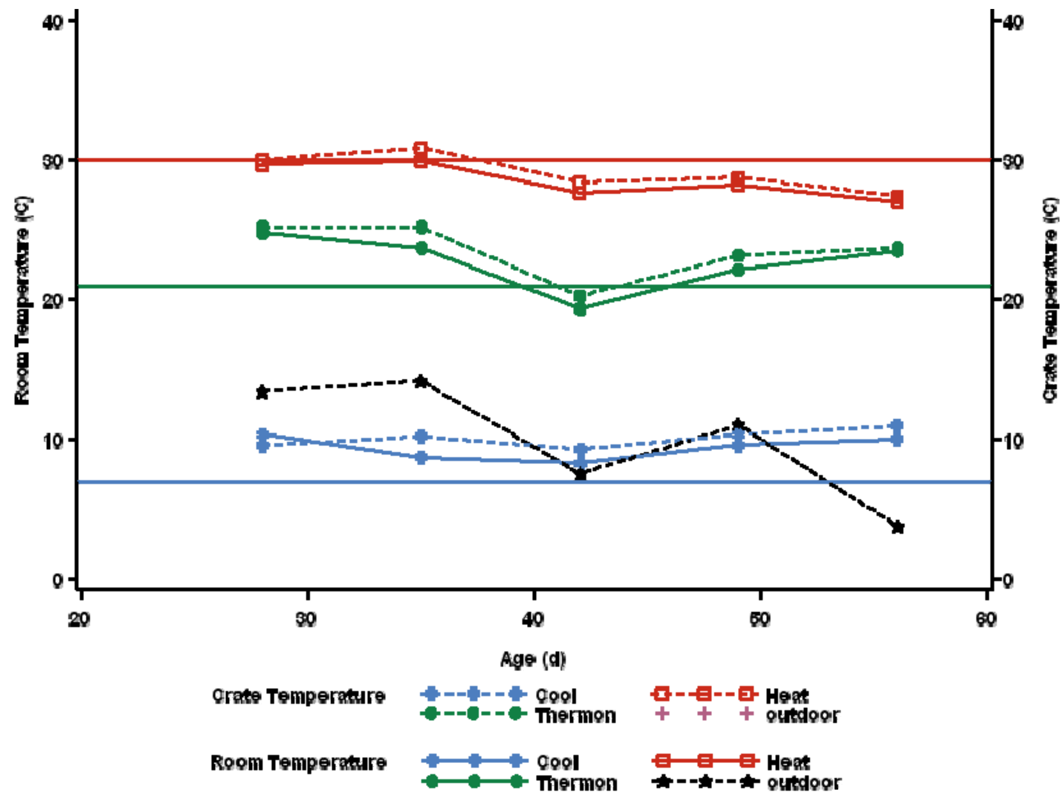

Figure 0.1. Target and average actual temperatures observed outdoors, inside environmental chambers and inside crates during a 10 h feed withdrawal period (8pm to 6am) for Ross 308 broilers at 28, 35, 42, 49 and 56 d of age.

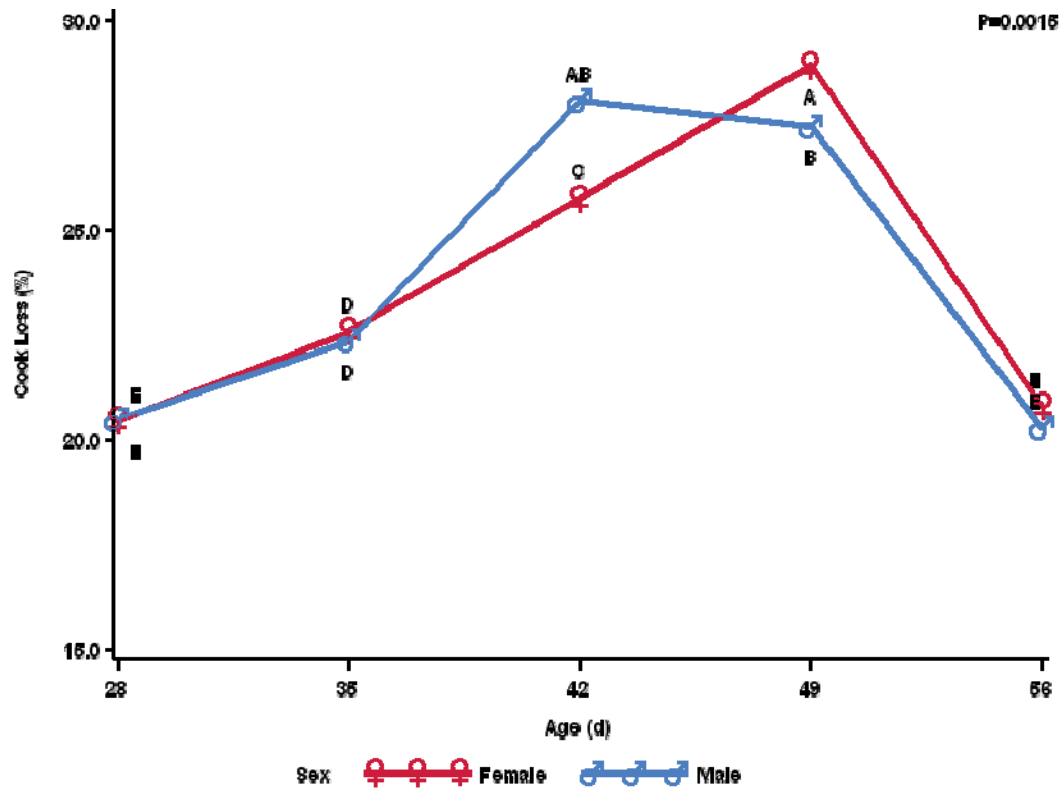

Figure 0.2. Interaction between bird age (28, 35, 42, 49 and 56 d) and sex on cooking loss of *Pectoralis major* at deboning in Ross 308 broilers. Points with no common letters do not differ significantly (P=0.0015).

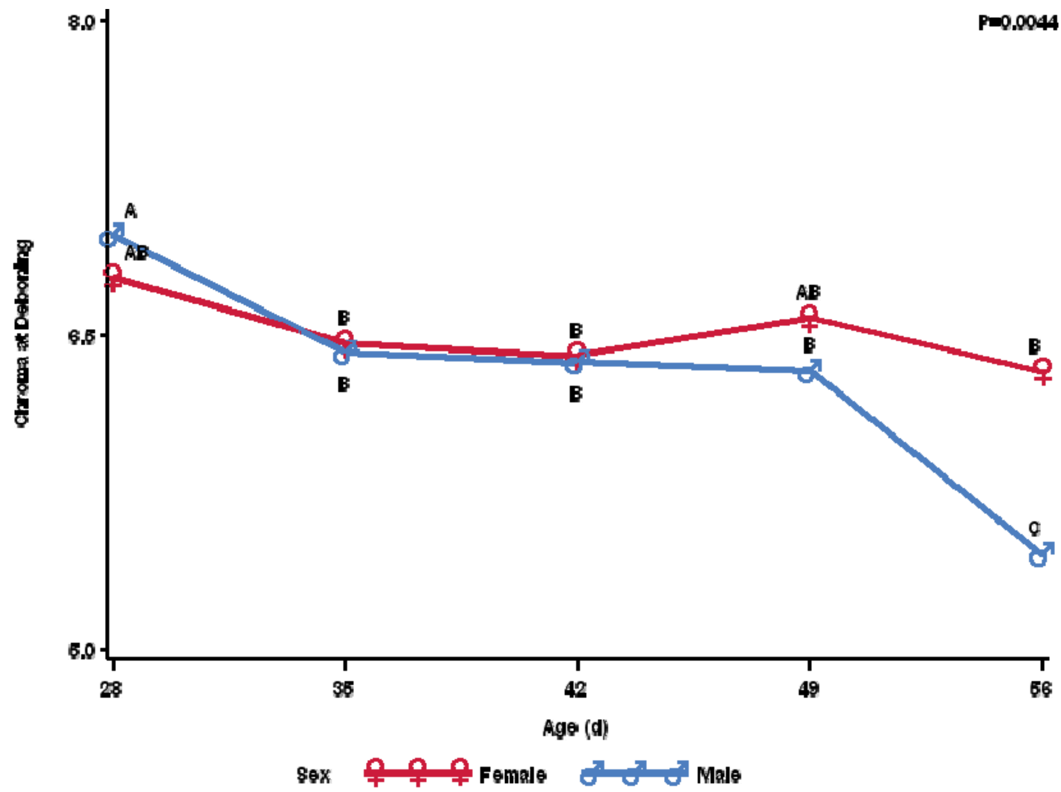

Figure 0.3. Interaction between bird age (28, 35, 42, 49 and 56 d) and sex on chroma of *Pectoralis major* at deboning in Ross 308 broilers. Points with no common letters do not differ significantly (P=0.0044).

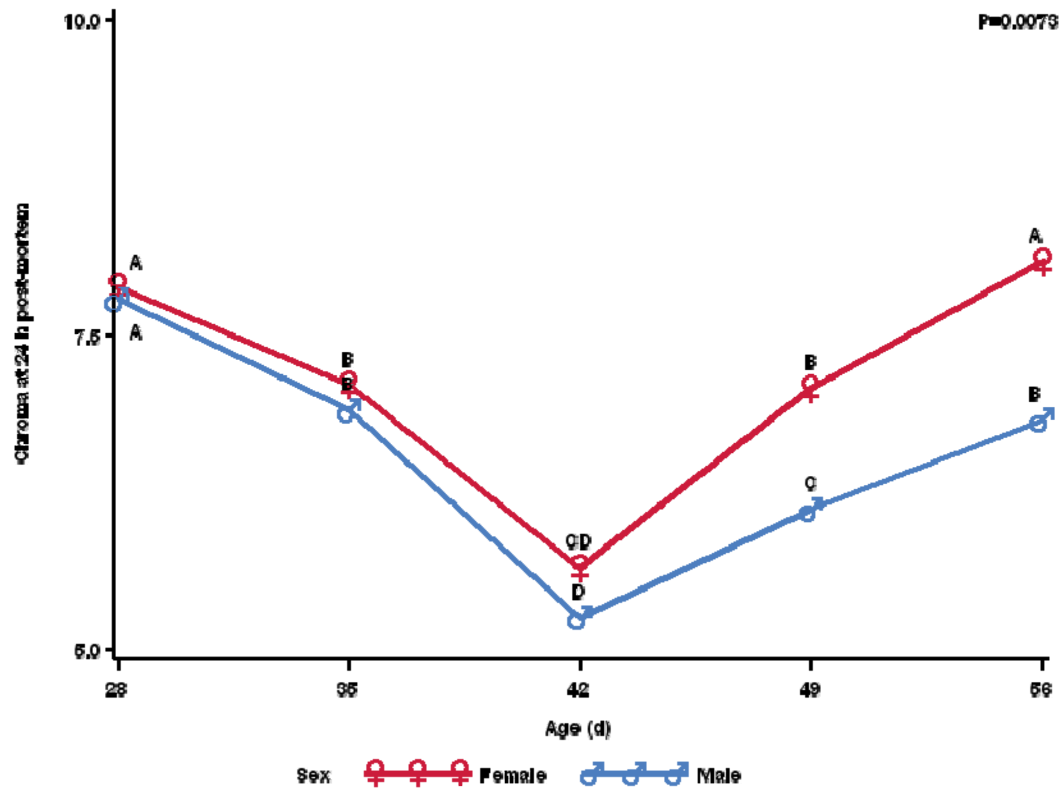

Figure 0.4. Interaction between bird age (28, 35, 42, 49 and 56 d) and sex on chroma of *Pectoralis major* at 24 h *post mortem* in Ross 308 broilers. Points with no common letters do not differ significantly (P=0.0073).

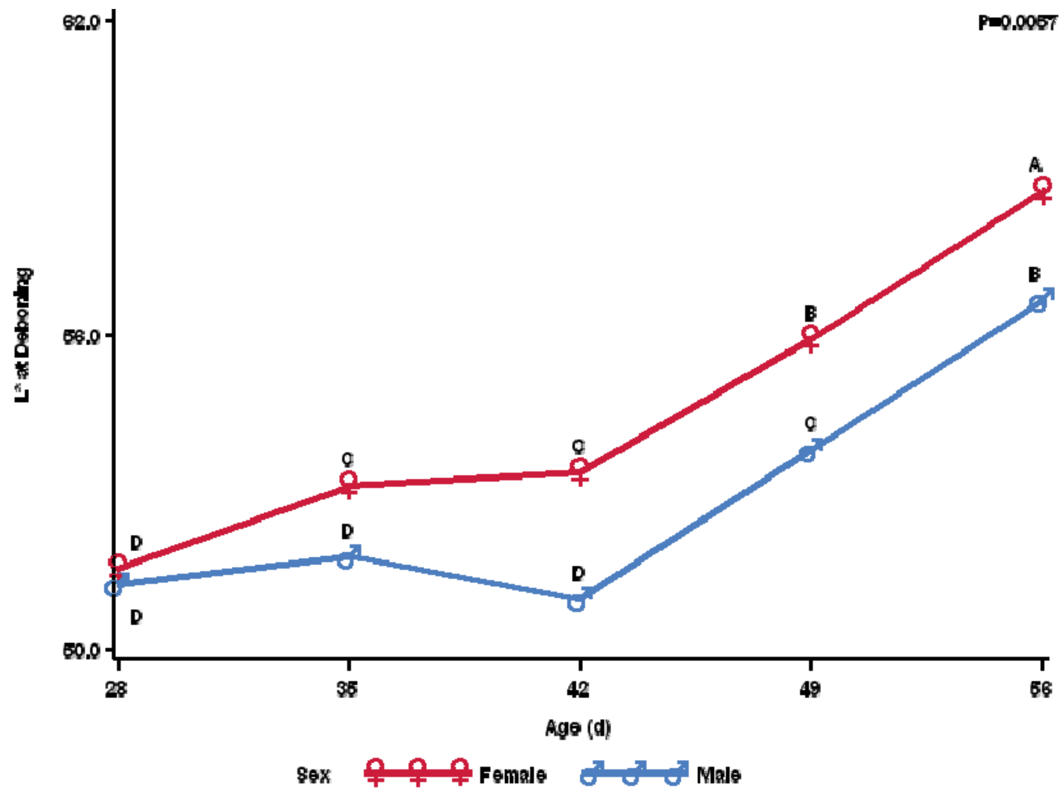

Figure 0.5. Interaction between bird age (28, 35, 42, 49 and 56 d) and sex on L* (lightness) of *Pectoralis major* at deboning in Ross 308 broilers. Points with no common letters do not differ significantly (P=0.0057).

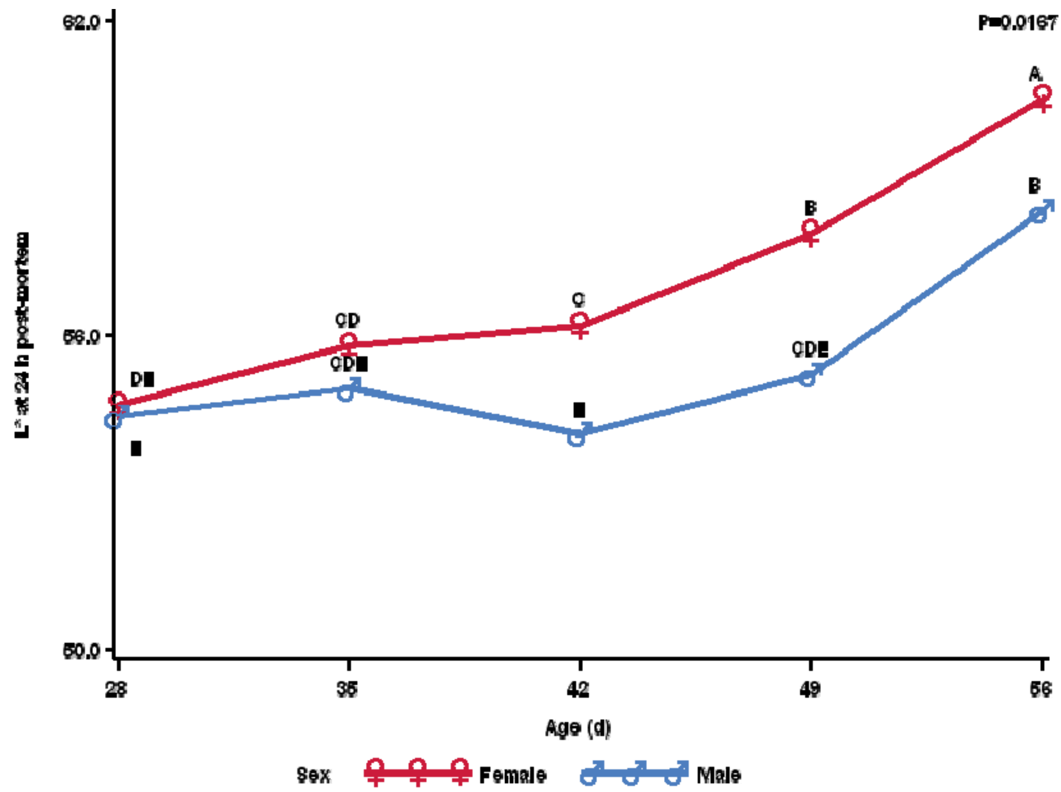

Figure 0.6. Interaction between bird age (28, 35, 42, 49 and 56 d) and sex on L* (lightness) of *Pectoralis major* at 24 h *post mortem* in Ross 308 broilers. Points with no common letters do not differ significantly (P=0.0167).

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3. Value-based pricing for poultry producers

Alberta broiler producers are paid based on the live weight of the birds they produce (Carney and Schneider, 2005). Basing prices on live weight alone can be detrimental to the overall efficiency of the supply chain since producers may choose to produce birds that are heavier than required by the processing industry. Furthermore, at the same live weight, differences in bird conformation, composition and functional properties can make a carcass more or less valuable to processors and consumers. A value-based pricing model would provide cues that would motivate producers to raise broilers that more closely match the requirements of the Alberta processing industry. However, a value-based pricing model would require the identification of quality factors that are valuable to processors and consumers and can be influenced by producers on the farm.

This thesis has described several factors that affect poultry product quality. One of the most substantial costs in poultry production is feed. Producers may choose to minimize costs by altering the nutrient or ingredient composition of the diets fed on their farm. Ingredient choice can affect the color of poultry meat; birds fed diets with a high proportion of corn tended to have more yellow breast meat. Protein and energy level profoundly affected the composition of eviscerated carcasses but did not affect the composition of breast meat at 52 d of age. The interactions between dietary protein and energy level on carcass composition traits are complex. Low energy and excessive protein resulted in low carcass ash and protein and increased fat. Similar results were observed with low dietary energy and very low dietary protein. These results must be considered in light of the effect of these treatments on growth and yield. Abdominal fat pad deposition was highest in birds fed very high levels of dietary protein (115% of recommended levels).

Limiting dietary energy appeared to limit growth between 21 and 28 d due to digestive tract or body size limitations; however, by the end of the experiment (56 d) these birds had caught up with the other groups. If broilers are to be marketed at earlier ages restrictions in growth during this period could represent a lost opportunity for producers; although low energy diets may cost the producer less money, the loss of revenue due to reduced growth could offset these savings.

Effects of dietary energy and protein on morphology of broiler breast muscle were found to be limited; the size of the bird and age had the greatest influence on the shape and dimensions of *P. major* muscle. The 12 to 14 h period prior to slaughter has been identified as a major source of quality problems for broiler meat. Holding birds at cooler temperatures during feed withdrawal resulted in improved functional properties; namely, higher ultimate pH and decreased drip loss and Allo-Kramer shear values. Exposure to heat stress conditions resulted in lower pH, higher drip loss and Allo-Kramer shear values. Careful

management of this period by minimizing exposure to heat stress conditions would result in more valuable carcasses at the processing plant.

Although producers cannot control what happens to their birds once they have left the farm, stress associated with shackling appears to modify color properties but not ultimate pH, drip or cooking losses. Shear values were actually higher in birds that were prevented from struggling prior to slaughter.

Age at marketing is largely out of producers control; marketing dates are scheduled by the processing plant. The results of the studies described in this thesis indicate that older broilers have a higher proportion of carcass protein, lower ultimate pH, drip loss, cook loss and lighter meat. Shear values increased with age up to 56 d, but remained under thresholds that are considered tender. It would appear that broilers marketed at later ages would be more valuable due to their superior functional properties and composition.

Most interestingly, this thesis has identified gender as a major contributor to the functional properties of broiler meat. Females consistently had lighter breast meat and higher drip loss than males. Ultimate pH was numerically lower in females in the nutrition study, and was statistically lower in the handling experiment. The metabolic reasons for these differences require further study, but could be exploited to improve the efficiency of the broiler supply chain.

In conclusion, results from research indicate that:

- Low energy diets in combination with either very low or very high protein have unfavorable effects on carcass composition
- Dietary energy and protein levels do not appear to influence functional properties of breast meat enough to have commercial significance.
- Dietary energy and protein levels do not appear to influence breast muscle morphology.
- Size and age of the bird have the greatest influence on breast muscle morphology.
- Functional properties of breast meat appear to improve with age
- Broiler sex appears to have a far greater influence on functional properties and color than previously reported.
- Exposure to cool temperatures (9C) during feed withdrawal improved the functional properties of breast meat
- Shackling duration affected color properties and tenderness values but did not alter functional properties

3.1 QUALITY FACTORS THAT MAY BE INFLUENCED ON FARM

Based on the results detailed in this thesis, the following factors may be influenced by producers:

- Carcass composition via dietary protein and energy levels

- Meat color via ingredient choices
- Functional properties via environmental temperature during feed withdrawal
- Functional properties and meat color by sex-separate rearing

3.2 FUTURE CONSIDERATIONS

- Determine if male and female broilers have different levels of muscle glycogen.
- Determine if muscle glycogen content changes with age.
- Determine the role of AMPK and reactive oxygen species in *post mortem* metabolism in different broiler strains.
- Determine the role of AMPK and reactive oxygen species in *post mortem* metabolism in male and female broilers.
- The metabolic and biochemical causes of the differences between sexes in functional properties of broiler breast meat should be investigated.
- Investigate the extent of strain differences in breast muscle morphology and determine if some strains are more suitable for fast food templates.
- Determine the threshold temperature during feed withdrawal required for improved functional properties.
- Cost analysis of reducing temperatures during feed withdrawal to improve functional properties.

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