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Physical and Biochemical Factors Affecting Breast Fillet Tenderness in Broilers Reared For Divergent Market Demands

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Abstract

Market trends have dictated that broilers be reared for divergent market demands for decades. Also, broiler genetic strains have been adapted to meet market demands which include: genetic selection for improved breast meat, the practice of growing birds to older ages and greater market weights to meet demands, and decreasing postmortem (PM) aging time to improve processing efficiency and fillet yield. These production practices can also affect product quality, specifically boneless breast fillet tenderness and water-holding capacity. To address these factors, a series of experiments were conducted to determine the effect of strain and slaughter age on meat quality parameters, as well as potential causes of these differences in meat quality. Furthermore, commonly accepted methods for improving boneless breast fillet meat quality were evaluated for the effect of older slaughter age and greater market weights. Based on these results, it appears that, regardless of debone time, broilers reared to greater market weights are tougher than birds reared to a lighter market weight. There are several factors that are compounding this increased toughness, including slower progression of rigor development, greater myofibrillar diameter, and perhaps decreased PM fragmentation; however, the relationship among factors is not fully exploited. Also, it appears that boneless breast fillet marination is critical in these larger birds to alleviate the toughness associated with pre-rigor debone, and may be necessary even when birds are deboned at 24 h PM. Therefore, based on these results it can be recommended that longer PM aging prior to debone is necessary when birds are slaughtered at heavier market weights, and aging the meat off the bone may be beneficial to improving boneless breast fillet tenderness. Furthermore, the commercial practice of tumble-marinating is effective for breast fillets of greater size and may be necessary to avoid negative consumer perception of tenderness.

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Sara, Gary, Doug, and Rodney: I absolutely would not have completed this dissertation without your help.

Dedication

I dedicate this dissertation to my son, Haden. As I write this, I am holding his caterpillar.

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Chapter 1

Introduction

Defining meat quality is a complex task because many intermingling factors contribute to final product quality. Tenderness and water-holding capacity (WHC) are perhaps the most critical quality attributes for consumer satisfaction (Lyon et al., 1985; Morgan et al., 1991; Smith and Fletcher, 1988; Cavitt et al., 2005; Saha et al., 2009a), and several antemortem, as well as postmortem factors, will affect the tenderness of the meat. One primary antemortem factor that will affect broiler breast meat quality is stress prior to slaughter; however, broiler strain, gender, and age at slaughter could also significantly affect meat quality (Owens et al., 2000; Berri et al., 2001; Mehaffey et al., 2006; Brewer et al., 2012 a, b).

In the commercial broiler industry, market demands have given rise to two primary market ages (sizes) for broilers. The smaller of the two, the "fast-food" market typically requires a bird in the 4.5 to 5 pound range (approximately 40 d of age). Birds processed for this "small bird" market will typically be used for bone-in and boneless parts for fast food and quick-serve restaurants, and the National Chicken Council (NCC) projected in 2012 that 41% of the commercial broiler market was dedicated to meeting this market demand. In recent years, the domestic demand for broiler breast meat, breast meat further processed products, and the advancement of boneless breast fillet portioning technology has led to the emergence of the "big bird" market. The NCC projected that, in 2012, 48% of the broiler market was allocated to this "big bird" market for use in further processed products, and broilers for this market are typically raised to 8-plus pounds (approximately 60 d of age).To further meet these "big bird" market demands, strains of broilers have been selected for generations for improved breast fillet yield, resulting in "high-yielding" strains. These high-yielding broiler strains can obtain a breast yield reaching approximately 25% of ready-to-cook weight and can potentially have breast weights in excess of 2.6lbs (1.19 kg; Brewer et al., 2012c).

High-yielding strains were developed to increase the notoriously small profit margins of the commercial broiler industry and produce high quantity of the traditionally highly valued meat portions for the U.S. In addition to these genetic advances, however, the nature of poultry processing dictates that efficiency is maximized to maintain profitability. This can be accomplished by streamlining broiler processing and maximizing boneless breast meat yield. Because of this need, many commercial facilities are trending towards deboning carcasses immediately after leaving the chiller. At this point in processing, the carcass is approximately 2 h postmortem (PM) and, because the completion of rigor mortis in broilers is typically considered to be around 4 h PM, this often means deboning carcasses prior to the completion of rigor (Lyon et al., 1973; Stewart et al., 1984; Alvarado and Owens, 2005). This practice maximizes processes efficiency because carcasses are not stored for additional time prior to debone. Also, breast fillet yield will be improved through decreased drip loss (Hirschler and Sams, 1998) and decreased PM proteolysis; much of the PM proteolysis that will cause the muscle to tear leaving remnants of meat on the carcass has not occurred at 2 h PM (Sams, 1999; McKee et al., 1997). Although efficiency is improved by deboning breast fillets early PM, there can be an effect on breast meat quality also as a result of this practice (Lyon et al., 1985; Papa and Fletcher, 1988).

Even though meat quality can be drastically affected prior to the animal reaching the processing facility by effecting muscle metabolism (antemortem), much of what will decide final product quality will occur PM, primarily, the development of rigor mortis. Early deboned breast meat will be tougher than broiler breast meat aged to after the completion of rigor (Lyon et a., 1985; Stewart et al., 1984; Papa and Fletcher, 1988; Cavitt et al., 2005; Mehaffey et al., 2006; Brewer et al., 2012a, b). This toughness is traditionally attributed to shorter sarcomere length.

Contraction of the sarcomere will occur during debone, which stimulates the muscle to contract, while there is still sufficient energy present in the muscle (Nakamura et al, 1975; Herring, 1967). Recent research has also indicated that birds from high-yielding strains and birds reared to older ages have the potential to be tougher than birds from standard yielding strains and birds reared to younger ages (Berri et al., 2001; Mehaffey et al., 2006; Brewer et al., 2012a,b). So, although high-yielding strains and early debone have the potential to improve profitability, product quality could potentially be sacrificed from the increased toughness that has been attributed to these factors.

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Chapter 2

Literature Review

Muscle Contraction

Muscle contraction and relaxation have been reviewed by several authors (Greaser and Gergely, 1971; Offer et al., 1989; Lawrie, 1998; Alvarado and Owens, 2005) Energy, in the form of ATP, is required for both muscle contraction and relaxation. During contraction, ATP is required for the energy to tilt the myosin head which will pull the actin toward the center of the sarcomere, resulting in the contraction and shortening of the sarcomere. This begins when the motor end plate receives an action potential from the motor neuron, causing an influx of K^+ and an efflux of Na^+ from the membrane of the muscle cell, also called depolarizing. When the polarity of the muscle reaches a threshold of -70mV the action potential is propagated down the muscle fiber, through the t-tubule system to the sarcoplasmic reticulum. The stimulated sarcoplasmic reticulum releases Ca^{2+} into the sarcoplasmic fluid. Free Ca^{2+} in the sarcoplasmic fluid then binds to one of the regulatory components of the actin fiber, troponin C. This binding sets a chain of reactions into action; a change in the configuration of troponin-I releases the inhibition of tropomyosin exposing the myosin binding site on actin allowing an actomyosin bond to form. Finally, hydrolysis of ATP causes the tilt of the myosin head sliding the actin toward the center of the sarcomere (Huxley and Hanson, 1954). During muscle contraction, actomyosin bonds are continually being formed and broken, and ATP is required for the dissociation of actomyosin bonds also.

During relaxation, ATP is required to dissociate the final actomyosin bonds, to pump the Ca^{2+} back into the sarcoplasmic reticulum, and to repolarize the membrane by active transport of K^+ out of the cell and Na^+ into the cell. By pumping Ca^{2+} back in the sarcoplasmic reticulum, Ca^{2+} concentration in the sarcoplasmic fluid decreases from approximately 10^{-5} to 10^{-7}. At this

decreased concentration, troponin-C will no longer cause the change in conformation of troponin-I and actin and myosin bonding will be inhibited.

Muscle Metabolism and Rigor Mortis

Rigor mortis, Latin for "stiffness of muscle" is the biochemical change that occurs in the muscle postmortem (PM) in the conversion of muscle to meat. As the animal is exsanguinated during slaughter, muscle metabolism switches from aerobic metabolism to anaerobic metabolism (Pearson and Young, 1989). During aerobic glycolysis and oxidative phosphorylation large quantities of ATP (37 ATP produced/1 glucose molecule) are derived from the oxidation of nutrients in a complex set of reactions ending with the electron transport chain (Alvarado and Owens, 2005). During the first (delay) phase of rigor, the muscle will still be extensible as oxygen depletion occurs and the muscle switches from aerobic to anaerobic metabolism. During anaerobic glycolysis, however, energy is derived from the glycogen stores in the muscle. Glycogen depletion in the muscle marks the onset of rigor; when deboning meat during the onset phase of rigor, the stimulus of cutting the muscle will result in myofibrillar contraction and when the muscle is removed from its skeletal restraints it can contract significantly (Papa and Fletcher, 1988). As glycogen stores become depleted during the onset of rigor, muscle pH declines to approximately 5.9 due to the accumulation of lactic acid and the loss of circulatory system to remove it from the muscle; when muscle pH reaches this level this marks the onset of rigor, and the inhibition of glycolysis (Sams, 1999). The conversion of pyruvate to lactic acid is the final step of anaerobic glycolysis. In aerobic glycolysis, this pyruvate will not be converted to lactic acid and will enter the citric acid cycle where energy metabolism will continue; however, this cannot occur without O_2 present in the system. Generation of ATP will diminish due to the inhibition of glycolysis during this phase, and permanent actomyosin bonds will begin to form

when ATP levels reach 1µM/g (Hamm, 1960), and permanent actomyosin bonds cause the muscle to become rigid (Greaser, 1986; Faustman, 1994).

Rigor mortis development can be evaluated by measuring the PM muscle pH. Resting muscle pH of an animal prior to slaughter is slightly alkaline, approximately 7.4 (Newbold and Harris, 1972). During the completion of rigor mortis the accumulation of lactic acid in the muscle will cause the pH to decline to an ultimate pH of around 5.4 to 5.7 (Hedrick et al., 1994; Murray, 1995).

During the resolution of rigor, the final stage, proteolytic enzymes will breakdown the structural integrity of the myofibrillar proteins which can result in some late PM tenderness. The calpain proteolytic system is largely responsible for this proteolytic digestion and will be discussed later in this review. In poultry, this proteolytic digestion occurs between 6 to 8 hours PM (Hedrick et al., 1994).

Meat Quality

Meat product quality can be dependent on many different factors, but the most important factors include water-holding capacity (WHC) and tenderness (Lyon et al., 1985; Morgan et al., 1991). Many factors can affect WHC and tenderness, including pH (rigor development), protein functionality, fat content, and collagen content (Allen et al., 1998).

Postmortem pH

As previously mentioned, pH can be measured to track the progression of rigor. Also, rate of pH decline, as well as, ultimate pH can affect meat quality, specifically, rapid PM pH decline. If this pH declines rapidly PM, while the temperature of the meat is still high, the resulting condition is known as pale, soft, and exudative meat (Hedrick et al 1994; Murray, 1995). Pale, soft and exudative (PSE) meat is characterized by its light color, poor WHC, soft

texture, and poor functionality in further processed products (Lawrie, 1998; Owens et al., 2000; Woelfel et al., 2002). These characteristics are due to extensive protein denaturation resulting from rapid PM glycolysis and pH decline while the temperature of the meat is still high. This denaturation of myofibrillar proteins is what dramatically reduces the functionality of the proteins, and results in the poor WHC and the soft cooked meat texture associated with PSE. Additionally, the characteristic light color (L*>54; Woelfel et al., 2002) is a result of denatured proteins and less water held in the bound and immobilized phase; thus, causing greater reflection of light. It has been suggested that increased rate of chilling for PSE-prone birds may minimize the effect of this condition (Dransfield and Sosnicki, 1999). The exact cause of PSE in poultry is unknown; however, in pork, the condition is related to a mutation of the ryanodine receptor, causing leaking of $Ca^{2+}$from the terminal cisternae into the interstitial space around the myofillaments, resulting in increased stimulation and exhausting glycogen supplies more quickly (Fuji et al., 1991; Sams et al., 1999).

As previously mentioned, rapidly chilling of PSE prone carcasses can reduce the protein denaturation associated with this condition (Dransfield and Sosnicki, 1999). Carcass chilling slows the progression of rigor mortis. Kijowski et al. (1982) observed pH values from broiler carcass that were deboned at 0.25, 0.50, 1, 2, 4, 8, and 24 h PM, and noted a rapid pH decline up to 2 h PM, with no significant decline in pH beyond 2 h PM. Also, the ultimate pH noted in this study was 5.75, slightly higher than traditionally expected 24 h pH values. However, in this study, carcasses were "hot-boned" or stored in a cooler and not immersion chilled to an appropriate end point temperature prior to debone.

In contrast to PSE, is another condition known as dark, firm and dry (DFD). This occurs when the pH of the meat remains high throughout the rigor process, due to excessive antemortem

glycogenolysis before slaughter. Dark, firm, and dry meat will have higher than average WHC, although it will appear dry on the surface, and darker than expected color (Hedrick et al., 1994). The difference in WHC between these two biochemical conditions can be explained by the ultimate pH of these conditions. For meat, the isoelectric point (pI) is in the range of 5.0 to 5.4 (Hamm, 1986), and, at this pH, there is no net charge on the muscle proteins (Pearson and Young 1989; Hendrick et al. 1989). Therefore, lower final pH, closer to the pI, results in decreased WHC, whereas higher ultimate pH, farther away from the pI, will improve WHC (McKee and Sams, 1997; Owens et al., 2000).

Research has indicated that bird size can impact rate of pH decline in broilers. Remingnon et al. (1993) concluded that the *Pectoralis major* of commercial broilers is entirely composed of glycolytic fibers (fast-twitch, fast fatiguing) which do progress through rigor at a slower pace than less glycolytic fibers (slow-twitch, slow fatiguing). It has also been concluded that birds selected for growth and breast fillet yield had slower rate of *Pectoralis* pH decline PM, which corresponded to lower glycolytic potential of the larger breast muscles. Despite lower glycolytic potential, there was no effect on metabolic pathways between the strains evaluated, and the decreased rate of pH decline does not impact breast fillet color or WHC (Berri et al., 2001; Bihan-Duval et al., 2001). Cavitt et al. (2005) reported that within one commercial strain, there was difference in rate of pH decline between genders; however, the birds used in this study were the same age and also within 200 g of target slaughter weight. Mehaffey et al. (2006) conducted a study using 5 different commercial strains with differing yields but failed to note a consistent relationship between pH decline and breast yield. When comparing high-yielding and standard-yielding commercial strains, Brewer et al. (2012) concluded that breast fillets from birds with greater body weight at slaughter did progressed through rigor at a slower pace, in

agreement with Berri et al. (2001) and Cooper and Fletcher (1997). The common factor among these studies is the difference in body weight at slaughter, with the birds with the heaviest live weights showing a slower rate of pH decline. Berri et al., (2001) and Bihan-Duval et al., (2001) attributed this difference in pH decline to the rate of glycolysis in larger muscles.

Water-holding Capacity

Tenderness, juiciness, and color are all meat quality parameters closely linked to WHC (Jeffery 1983). As previously mentioned, ultimate pH can significantly affect WHC because of the low pI of the muscle proteins. As previously mentioned, PSE meat will have characteristically low WHC, while DFD will have characteristically high WHC; these conditions are classic examples of the effect of meat pH on WHC.

Water-holding capacity can be separated into two separate functional effects. These effects are the ionic effect and the steric effect (Alvarado and Owens, 2005). If there is a net charge on the muscle proteins, water will be attracted to and held in the muscle, this is the ionic effect. However, near the isoelectric point, when there is no net charge on the muscle protein there is less attraction of water therefore less water is held in the muscle. Also, when there is not net charge on the muscle protein there is no charge repulsion of the muscle fibers. With no charge repulsion there is less interstitial space for water to be held, this is the steric effect. Therefore, although slightly different they are very closely related and the ionic effect does impact the steric effect.

Previous research has indicated that strain has very little effect on WHC of broiler breast fillets (Bihan-Duval et al., 2001; Mehaffey et al., 2006; Lopez et al., 2011; Brewer et al., 2012a, 2012b) despite differences in pH and potential difference in glycolytic potential. The contractile state of the muscle, however, can affect WHC. Shorter sarcomeres will result in less interstitial

space for water to be held, decreasing the steric effect of WHC (Hamm, 1986; Lawrie 1998). Brewer et al. (2012 a, b) illustrated this effect by showing decreased WHC for broiler breast fillets deboned at 2 h PM compared to breast fillets deboned at 4 and 6 h PM. Furthermore, among strains, differences in shear force are not necessarily caused by or correlated with cook loss because, in this study, there were no difference in cook loss between strains at any debone hour.

Tenderness

Postmortem Aging

Tenderness is the most important factor for consumer acceptance of meat products. Post-mortem aging time has the single greatest impact on broiler breast tenderness. Stimulation of the muscle, prior to the completion of rigor, by deboning will cause the muscle to contract and, without the skeletal restraints and energy to dissociate actomyosin bonds, the muscle will remain in a contractile state (Stewart et al., 1984; Papa and Fletcher, 1988). Because of this, samples deboned prior to the completion of rigor mortis will result in increased shear force values and objectionable sensory tenderness ratings compared to broiler breast fillets aged on the bone until after the completion of rigor (Lyon et al., 1985; Dawson et al., 1987; Thompson et al., 1987; Xiong et al., 2006, Cavitt et al. 2005; Mehaffey et al., 2005; Brewer et al., 2012a, b). Toughness in broiler breast fillets is traditionally attributed to increased overlap of the thick and thin filaments (actin and myosin) resulting from shortened sarcomeres (Locker, 1960; Herring et al., 1967; Papa and Fletcher 1988), especially in animals that are slaughtered at young ages, such as commercial broilers (Nakamura et al, 1975). Because of this potential for PM sarcomere contraction, it has been recommended that broiler carcasses be aged from 4 to 6 hours PM (after the ATP depletion) before they are deboned (Papa and Lyon, 1973; Stewart et al., 1984).

Although sarcomere shortening is traditionally thought to be the primary cause of toughness boneless breast meat, several other factors could potentially contribute to toughness of breast fillets from birds grown to older ages and yielding more. These factors include: decreased rate of rigor mortis, increased myofibrillar diameter, and decreased PM myofibrillar fragmentation.

Rigor Mortis Progression

It has been concluded that larger birds progress through *rigor mortis* at a slower pace due to slower glycolytic activity in the muscle. Thus, there could potentially be sufficient energy in the muscle to cause contraction at later PM debone times. Brewer et al. (2012b) found that broilers that yielded significantly more reached equivalent tenderness at 6 h PM as broiler that yielded less did at 4 h PM. Moreover, Wakefield et al. (1989) concluded rapid cooling of slower glycolysing muscles will increase toughness, and Dransfield and Sosnicki (1999) stated carcasses progressing though rigor more slowly should be chilled more slowly to minimize toughness. Therefore, the rate of pH decline could have two-fold effect on tenderness: 1.) sufficient energy in the muscle late PM to cause contraction; 2.) too rapid rate of chill while pH is still high resulting in Ca^{2+} leak from the terminal cisterne stimulating contraction, also known as cold shortening (Sams, 1999; Hedrick, 1994).

Muscle Fiber Diameter

In red meat species, it has been suggested that increased fiber diameter can cause increased shear force of meat (Tuma et al. 1962; Crouse et al. 1991; Shakelford et al., 1994). Generations of selection for increased breast fillet yield in the commercial broiler has increased the broiler breast muscle size significantly. Previous research has indicated muscle fiber number is fixed prior to hatching in broilers; therefore, as the bird ages, the increasing breast fillet yield

cannot be attributed to post-hatch hyperplasia (increased number) of muscle fibers (Mitchell and Burke, 1995; Henry and Burke, 1998). It has also been suggested that satellite cells could play a role in increased fillet size (Acar et al., 1993); however, results investigating this have been inconclusive and estimation error of satellite cells is large (Campion et al., 1982) Instead, it is commonly accepted that hypertrophy (increase in fiber diameter), as well as elongation of the muscle fiber, is the reason for increased broiler breast fillet weight as birds age (Smith 1963; Hedrick, 1994). Among strains with differing yields, however, there have been conflicting reports regarding fiber diameter. Acar et al. (1993) found no difference in myofibrillar diameter and hypothesized that increased myofibrillar number was the cause of increased breast fillet yield between commercial strains. Similarly, Scheuermann et al. (2003) observed no difference in muscle fiber diameter (MFD) between strain crosses even if there was a difference in fillet yield, and attributed the fillet yield difference to greater myofibrillar number (MFN). Conversely, Remington et al. (1993) demonstrated a difference in fiber diameter between fast- and slow-growing strains with differing fillet yield slaughtered at the same age. Furthermore, it is commonly accepted that fiber diameter will be significantly smaller in birds not selected for breast fillet yield, such as commercial leghorns (Aberle et al., 1979). Therefore, increased yield appears to be closely associated with increased muscle fiber hypertrophy, particularly between ages; however, there is little data on the effect of MFD on breast meat tenderness, and increased fiber diameter could be contributing to increased toughness of boneless breast fillets, especially in birds reared to greater market weights.

Myofibrillar Fragmentation

During the resolution of rigor, muscle proteolysis can occur which can improve meat tenderness. In red meats it is commonly accepted that PM proteolysis plays a significant role in

improving meat tenderness (Hedrick, 1994). It has been shown that the calpains are responsible for PM proteolysis, and improved cooked meat tenderness primarily through degradation of the Z-disks and desmin (Koohmaraie, 1994). In poultry, it is also suggested that a general weakening of the myofibrillar lattice, not necessarily the degradation of the Z-disc proteins, is responsible for increased PM tenderness 24 h post-slaughter. Regardless of exact mechanism, however, increased fragmentation of the myofibril with longer periods of storage postmortem is evidence of this proteolytic digestion (McKee et al., 1997).

Research conducted on the calpain proteolytic system has surrounded the enzymes μ-calpain, m-calpain, and the endogenous inhibitor of these, calpastatin. Calpastatin is responsible for regulation of the calpain proteolytic system (Koohmaraie et al., 1988), and it has been suggested that increased calpastatin activity could result in increased toughness in meat (Whipple et al., 1990). Both m- and μ -calpain, named because of the Ca^{2+} concentration required for activating these proteases, and calpastatin are responsible for the normal regeneration, and building of skeletal muscles (Goll et al. 1992). Furthermore, Shakelford et al. (1994) concluded that cattle that had greater meat yield also had greater calpastatin activity in the muscle, and increased shear force compared to animals that yielded less.

It is the nature of the proteases that they undergo autolysis in the presence of Ca^{2+}; therefore, Ca^{2+} activates these proteases and also leads to autolysis and eventually inactivity (Koohmaraie et al., 1988). In PM muscle, m-calpain levels remain relatively constant through 24 h PM; however, μ-calpain levels decrease drastically indicating activation and subsequent autolysis (Veiseth et al., 2001). Because of this, autolysis and inactivation, it has been concluded that μ-calpain, not m-calpain, is responsible for PM tenderization (Koohmaraie, 1988). Walker et al. (1995) reported that μ-calpain in avian breast muscle was inactive by 24-h PM. In broilers,

there appears to be some benefit to holding boneless breast fillets for aging periods greater than 24 h PM to improve breast fillet tenderness. Lyon et al. (1992) reported that holding fillets up to 24 h PM had very little effect on broiler breast fillet tenderness when fillets were deboned at 2 and 3 h PM; however, McKee et al. (1997) showed improved tenderness after 23 and 71 h PM, attributed to increased myofibrillar fragmentation and activity of the calpain proteolytic system.

Effect of Strain on Tenderness

High-yielding broilers have a different growth curve than standard strains used for a small bird market (Brewer et al., 2012c, d). Also, these high-yielding strains are typically reared to older ages; however, as the name would indicate, even at equivalent ages, these high yielding strains consistently produce greater breast yield compared to standard-yielding strains. Scheuermann et al. (2003) demonstrated that breast fillet depth was correlated with breast fillet yield, whereas, Lubritz (1997) stated that increased thickness of the fillet contributed 7-times more to increased fillet yield than an equivalent increase in fillet length or width. Furthermore, Brewer et al (2012b) reported a correlation between fillet depth and fillet yield and fillet depth and tenderness; so, fillets that were thicker at the cranial portion came from higher yielding strains, and these strains had significantly greater shear force values when birds were slaughtered at 60 d of age. Moreover, high-yielding broiler strains have tougher boneless breast fillets than broiler strains that yielded less even when breast fillets were deboned at 4 h PM (Brewer et al., 2012b). Mehaffey et al. (2006) also observed differences in yield between strains at both 2 and 6 h PM, and there were also differences found in tenderness between strains; however, there was no data published to suggest a correlation between tenderness and yield.

Brewer et al. (2012a) reported a difference in tenderness at 2 h PM among strains reared to 40 d of age. These differences in tenderness at 2 h PM, again, cannot be explained by pH or

cook losses which were not different among the strains used in this study; yet, there was no difference in breast fillet tenderness of broilers slaughtered at 40 d of age when deboned at 4 or 6 h PM, perhaps indicating earlier conclusion of rigor compared to older birds. Similarly, Lopez et al. (2011) reported no difference in shear force between broiler breast fillets from different strains slaughtered at 42 d and deboned at 4 h PM.

Effect of Age on Tenderness

Research conducted on age at slaughter has produced varying reports regarding the degree to which age at slaughter affects boneless breast fillet texture. Poole et al. (1999) conducted a study evaluating texture of male and female commercial broilers slaughtered at 5, 6, 7, and 8 weeks of age, and concluded that breast fillets from 5-week-old birds processed had lower shear force values than all other ages processed. In this study, it should be considered that fillets were deboned at 24 h PM, and therefore there would be little effect of rate of rigor development on shear values in this study.

Northcutt et al. (2001) slaughtered birds at 37, 39, 42, 44, 46, 49, and 51 days of age and deboned the breast fillets at 0, 2, 4 or 6 h PM, and found that birds reared to 46, 49, and 51 d of age have greater shear force values when deboned at 0 and 2 h PM than the younger birds. However, at 4 and 6 h PM, there was no effect of age on boneless breast fillet texture; so the authors recommended that, if deboning breast fillet early PM, it was best to use a young bird (42 or 44 d of age) to avoid meat toughness associated with age.

Marination

It is estimated that greater than 50 % of the commercial broiler market will be marinated prior to cooking (Smith and Acton, 2010). This is because marination can be used to increase yield, improve WHC (Zheng et al. 2000), and improve consumer acceptance of pre-rigor

deboned fillets by alleviating tenderness and improving flavor (Saha et al., 2009a; Lopez et al., 2012). Marination can be accomplished in several ways and with a variety of functional ingredients; however, most commonly, boneless fillets are vacuum-tumbled in a brine solution of water, salt, and phosphate. It is the function action of salt and phosphate in the marinade that increase WHC and improve tenderness (Hamm, 1960).

Actin and myosin are salt soluble in concentrations greater than 1% (Smith, 2010). By exposing these proteins to the brine solution, they will swell and unwind from their tight myofibrillar lattice, and become available for binding water. This is accomplished by disrupting the electrostatic interactions between protein molecules (Offer and Knight, 1988), and reducing the isoelectric point of the meat as the Cl^- ions interact with the myosin molecules (Hamm, 1986). Although adding greater amounts of salt to a meat product could continue to improve WHC and texture of meat products, salt is flavor restrictive and excessive salt concentrations can reduce palatability of the product (Alvarado and McKee, 2007; Smith, 2010). Saha et al., (2009b) when evaluating varying salt levels on consumer acceptance of boneless broiler breast fillets, Saha et al. (2009b) found no difference in consumer acceptance for salt levels ranging from 0.5% to 1%; however, at 1% salt, 20% of consumers thought breast fillets were too salty, whereas, at 1.25% salt 26% of consumers thought samples were too salty. In this study, it was concluded 0.75% salt was Just About Right for marinated breast fillets. Lopez et al. (2012) conducted a very similar study and reached very similar conclusions; yet, the higher salt percentages (1.0 and 1.25%) were not considered too salty, and resulted in the highest hedonic scores among panelists.

Phosphate works primarily to improve WHC by increasing the pH of the muscle, moving it farther from the isoelectric point (Hamm, 1972), and has been shown to improve cooked yield

(Smith and Young, 2007). Phosphates have been shown to have very little effect on improving the tenderness of boneless fillets (Alvarado and Sams, 2004). However, phosphates do have a synergistic effect with salt, because as the pH increases away from the isoelectric point with the addition of phosphate, the proteins become more extractable allowing for greater salt function (Smith, 2010). In the previously mentioned studies, Saha et al. (2009) and Lopez et al. (2012), phosphate concentrations of 0.40% and 0.35%, respectively, of final product were used. Again, despite the percentage difference in salt concentration used there was very little difference in the results of these two studies. It has also been suggested that using alkaline phosphate solutions (pH 9 to 11) can improve functional properties of PSE meat (Alvarado and Sams, 2003; Gorsuch and Alvarado, 2010; Woelfel and Sams, 2001); previous reports on this effect have not been unanimous as to the degree of improvement; however, in all instances, WHC was not necessarily fully restored, but improved with the use alkaline phosphate marinades.

Vacuum-tumble marinating is the most commonly used method for marination application in the commercial broiler industry (Smith and Acton, 2010). Although it is widely used, vacuum-tumble marinating has had mixed reports of efficacy for several decades (Young and Lyon, 1997; Young et al., 2004; Smith and Young, 2007). Research has been fairly conclusive, however, that the tumbling action (even in an ambient environment), and not necessarily the vacuum, is critical for marination uptake, compared to still or soak method marinating (Chen, 1982; Heath and Owens, 1991; Young and Smith, 2004). Injection of marinade is another application method that is used in the commercial poultry industry, and involves a series of needles piercing the product and injecting functional ingredients. Injection can be a more uniform method for introducing marinade into the system because an exact amount is pumped through the needles into the product (Alvarado and McKee, 2007). For both

marination methods though, the muscle membranes are disrupted, or pierced, allowing for greater exposure of the myofillaments to functional ingredients. Most commonly injection is used for bone-in products, whereas tumble marination is used for boneless products (Smith and Acton, 2010).

Need for Research

Research has indicated that breast fillets from broilers reared to older ages (50-plus days of age), and breast fillets from some high-yielding strains raised to equivalent ages have higher shear force values compared to standard-yielding strains.

Toughness in boneless broiler breast fillet meat is commonly attributed to shortened sarcomere length; however, there could be other factors affecting tenderness in high-yielding strains raised to older ages. It could be that older broilers and high-yielding broilers progress through rigor at a slower pace than younger birds and standard-yield strains, and there may still be sufficient energy in the muscle to cause contraction at 4 h PM. Perhaps, hypertrophy of the muscle fiber resulting in increased fiber diameter is a contributing factor to increased toughness. Increased muscle accretion generally can be attributed to increased calpastatin activity in the muscle; therefore, decreased fragmentation of the muscle resulting from decreased calpain activity could potentially be a cause of increased toughness.

Moreover, traditionally accepted methods for improving broiler breast fillet tenderness, such as vacuum-tumble marination and tank aging, should be evaluated for their effect on larger fillet sizes. Therefore, the purpose of this research was to determine the degree to which these factors affect breast fillet toughness in high-yielding strains, and how tumble-marination is affected by breast fillet size.

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Chapter 3

Tenderness of broilers reared for divergent market demands and deboned at varying times pre- and post-rigor

V.B. Brewer, B. Potter, and C.M. Owens

Abstract

A study was conducted to evaluate the effect of age at slaughter on progression of rigor development and shear properties of modern commercial broilers. Commercial broilers (n=336) were slaughtered at 38 or 52 d of age and deboned at 7 times postmortem. Samples for *Pectoralis* pH were collected immediately post-debone for evaluation of rigor development, and Meullenet-Owens razor shear force (MORSF) and energy (MORSE) were used to evaluate fillet texture. Growth performance parameters were also evaluated to confirm the difference in bird size associated with different slaughter ages. The 52-d-old broilers had greater (P<0.05) live weight, ready to cook yield, fillet weight and fillet yield than 38-d-old broilers. Also, 52-d-old broilers had greater (P<0.05) fillet dimensions at all parameters measured compared to 38-d-old broilers. Between 38 and 52 d of age, there was a 91.3% increase in live weight, which resulted in a 159.5% increase in fillet weight, and increased fillet weight was largely attributed to fillet thickness. Regardless of bird age, pH declined (P<0.05) up to 6 h PM, but pH values were between 6 and 24 h PM. Conversely, pH was greater (P<0.05) for 52 d old broilers than 38-d-old broilers at all times PM, indicating that older broilers were progressing through rigor more slowly than younger broilers. Fillet texture for 38-d-old broilers declined (P<0.05) between 2 and 6 h PM, with no difference (P<0.05) noted for MORSE between 6 and 24 h PM. For the 52-d-old broilers, however, MORSE declined (P<0.05) between 2 and 3 h PM, but MORSE was similar (P<0.05) 3 and 24 h PM. Moreover, regardless of PM deboning times, fillets from 52-d-old broilers had greater (P<0.05) shear force values than 38-d-old broilers, and it required aging 52 d old carcasses an additional 3 h (to 6 h PM) to reach equivalent tenderness of fillet from the 38-d-old broilers deboned at 3 h PM. These results indicate that there is some other contributing factor aside from slower rigor mortis development which is contributing to boneless fillet

MORSE of older broilers. This difference in tenderness in the current study cannot be attributed to moisture, because there was no difference ($P<0.05$) in cook loss or cooked meat moisture for any bird age or debone hour combination that coincided with a difference in fillet texture. Yet, muscle fiber diameter was compared to those of 38-d-old broilers. Thus, this difference in fiber diameter appears to be contributing to the difference in fillet texture observed between the two slaughter ages.

Key words: moisture, MORSE, myofibrillar diameter, pH, rigor mortis, slaughter age, texture,

Introduction

Two divergent markets have developed to better meet consumer demands for further processed poultry products, and bird size at slaughter has been tailored to meet the product specifications of these two markets. To produce the birds for the small-bird, or fast food market, birds are slaughtered at younger ages (approximately 4 to 5 lbs.) for cut-up bone-in and boneless parts. The National Chicken Council (NCC) projected that 41% of the commercial broiler market was allocated these products (NCC, 2012). Broilers are slaughtered at older ages (approximately 7.5 lbs.) to meet the demand for further-processed products, specifically breast meat further processed products, and the NCC projected that 48% of the market in 2012 was allocated to meet demands for these products. Innovation of portioning technology has also allowed fillets from birds reared for the further processed products market to yield a variety of products from a single fillet, improving efficiency and yield per man hour, while also meeting consumer demands.

To maximize quality of boneless breast fillets, it is recommended that broiler carcasses be aged 4 to 6 hour before deboning (Lyon et al., 1973; Stewart et al., 1984). However, streamlining broiler processing offers several advantages for maximizing efficiency, and one

common way to streamline processing is to debone carcasses immediately after leaving the water immersion chill tank (1.75 to 2 h PM). Not only does this practice reduce the amount of storage space required for holding carcasses prior to debone, but often will also result in improved breast fillet yield. This practice of early deboning often results in removing the meat from the bone prior to the completion of rigor mortis which will cause shortening of the sarcomeres (Cross et al., 1981; Papa and Fletcher 1988; Cavitt et al., 2004), resulting in decreased tenderness and consumer preference of boneless breast fillet texture (Lyon et al., 1985; Cavitt et al., 2004, 2005).

Previous research has also indicated that broiler age at slaughter can affect boneless breast fillet texture (Poole et al., 1999; Northcutt et al., 2001). These authors determined that broilers slaughtered at younger processing ages (5 weeks) resulted in reduced shear force values compared to broilers slaughtered at older processing ages (8 weeks). In these studies, however, the effect of rigor mortis development and other potential causes of textural differences were not evaluated. Therefore, the current study was designed to evaluate textural differences caused by bird age at slaughter, and its effect on rigor mortis development, as well as the effect of muscle fiber diameter and sarcomere length, on textural properties.

Materials and Methods

To evaluate the effect of progression of rigor and debone hour on boneless breast fillet tenderness, 336 broilers were obtained from a local processor and deboned at 7 times postmortem (PM). Of the 336 birds, half were reared for a small bird market and slaughtered at 38 d of age, whereas the remaining half were reared for a big bird market and slaughtered at 56 d of age. Following an 8-to-12 hour period of feed withdrawal, all broilers were commercially processed at the University of Arkansas Pilot Processing Plant. Birds were slaughtered in seven

replicates with equal number of small and large birds per rep (n=48/rep). From each replicate, birds were allocated randomly to two separate debone times. Birds were low voltage stunned, scalded, picked, and eviscerated as described by Mehaffey et al. (2006). Following evisceration, carcasses were immersion chilled in two stages for a total dwell time of 115 minutes (pre-chilled, 13C for 15 minutes, followed by chilling at 4C for 100 minutes). One debone time occurred immediately post-evisceration (0.25 h PM); so birds did not undergo immersion chill, rather deboned fillets were chilled in cooler (later described). Post-chill birds were immediately deboned (2 h PM) or well packed and aged on ice until the appropriate postmortem deboning time (3, 4, 6, 8, or 24 h PM). Debone treatments were replicated twice (n=12 birds/replicate debone; n=24/age and debone hour), and deboning was completed by 4 trained and experienced individuals. Immediately post-debone the caudal tip of the left breast fillet was removed, frozen in liquid nitrogen, and stored at -80C for pH analysis. The right breast fillet was weighed for determining yield and then packed on ice and held until 24 h PM when they were frozen and stored at -20C.

Pectoralis pH was determined using the iodoacetate method described by Sams and Janky (1986). Briefly, frozen tissue was pulverized and homogenized in an iodoacetate solution, and pH of the homogenate was measured to evaluate rigor development.

Samples for muscle fiber diameter were also taken to evaluate the effect bird age on fiber diameter. Samples from fillet deboned at 24 h PM were used to avoid any potential changes in diameter due to sarcomere shortening early PM. Samples were cut, fixed in formalin, and stained using hematoxylin and eosin. Fiber diameters were measured using Olympus microscope (Model Bx50; Olympus Corp. of the Americas, Center Valley, PA), Optronix engineering scanner (Model DEI-470), and Image Pro Plus analysis software (Media

Cybernetics, Inc., Warrendale, PA). All measurements were taken using 20 x magnifications. Two replicate stains were made per sample and 5 replicate fibers were measured per replicate stain (10 fibers measured per sample).

To prepare samples for texture analysis, fillets were cooked on raised wire racks to and internal temperature of 76C in a convection oven (Cavitt et al., 2005; Mehaffey et al., 2006). Fillets were weighed before and after cooking for measuring moisture loss, and were individually wrapped and stored for texture analysis the following day. Meullenet-Owens razor shear method (MORS) was used for analyzing texture with 3 replicate shears taken per fillet (Cavitt et al., 2005; Mehaffey et al., 2006). After texture analysis, fillet dimensions and cooked meat moisture samples were taken. Dimensions (length, width, and thickness) were measured using calipers.

After texture analysis samples were wrapped in aluminum foil and stored overnight (2C). The day following texture analysis, duplicate samples from each cooked fillet were weighed and placed in a drying oven for 24 h in accordance to the procedure of Lee et al. (1990). After 24 h samples were re-weighed and moisture loss was calculated as a percent of pre-drying weight.

These data were analyzed as an incomplete block design; the individual bird was the experimental unit. Main effects in the current study were bird age and debone hour. Slaughter and debone hour replicate were analyzed for data interaction and excluded from further analysis for lack of significance. Data for debone hour were analyzed using the GLM procedure of SAS (SAS Institute, 2002) and means were separated using Duncan's Multiple Range Test with differences considered significant at $P<0.05$. For age effect, TTESTs were used to determine difference between ages within each debone hour, and differences were considered significant at $P<0.05$.

Results and Discussion

Broiler age was a main effect in this study, and differences in age can have a significant effect on growth performance measures. Therefore, the effect of processing age on live weight, yield and fillet dimension parameters is presented in Table 1. Not unexpectedly, birds reared to 52 d had greater ($P<0.05$) live weights, ready-to-cook yields, fillet weights, and fillet yields compared to birds that were reared to 38-d of age. The degree of change between growth parameters was evaluated in the current study as a percent of the 38-d value; so, there was a 91% change in live weight, which equated to a 159.5% change in fillet weight, between the two slaughter ages. Fillets from 52-d-old birds were larger than the 38-d-old birds, with the greatest change in the thickness measurements between the two ages for cranial, midpoint, and caudal thickness. Lubritz (1997) reported that fillet thickness had a 7-fold greater impact on breast fillet yield than other dimensions. In the current study, it does appear that an increase in breast fillet thickness had the greatest effect on breast fillet weight and yield compared to other dimensions, in particular fillet length and width which changes of 27.4 and 30.7%, respectively, between slaughter ages. These results indicate the extreme growth potential of the modern commercial broiler, as well as that a great deal of muscle accretion occurs between the two market ages evaluated in the current study.

Pectoralis pH was evaluated to estimate PM rigor development and is presented in Table 2. During slaughter anaerobic metabolism will be used to produce ATP until the depletion of glycogen in the muscle. As a result, muscle will become increasingly acidic due to the accumulation of lactic acid until an ultimate pH of approximately 5.6 to 5.7 is reached (Kijowski, et al., 1982; Hedrick, 1994). Postmortem rigor development can significantly affect fillet texture and it is well documented that deboning fillets prior to the completion of rigor will result in increased shear forces and decreased consumer acceptance of boneless breast meat (Stewart et

al., 1984; Lyon et al., 1985; Papa and Fletcher, 1988; Cavitt et al., 2005). In the current study, as would be expected due to the progression of rigor, pH declined as time prior to deboning increased for both bird ages evaluated. Within fillets of 38-d-old broilers, pH declined ($P<0.05$) each hour between 0.25 and 3 h PM, as well as between 4 and 6 h PM, but with no differences ($P<0.05$) noted between 3 and 4 h PM and from 6 to 24 d PM. On the other hand, for the 52 d old birds, pH declined ($P<0.05$) at each debone hour between 0.25 and 6 h PM in fillets from 52-d-old birds, with no difference between 6 and 24 h PM. Considering that the inhibition of glycolysis occurs at a pH of 5.9 (Sams, 1999), the 32-d-old broilers in the current study reached a pH of approximately 5.9 at 3 h PM, while the 52-d-old birds in this study did not reach this pH until 4 h PM. Therefore, these results indicate there could be sufficient energy in the muscle at later PM times with larger birds to cause contraction of the myofibril upon deboning. The 24-h pH for both bird ages was within a normal range and at a level typically accepted to be the ultimate pH of muscle post-slaughter. With the exception of the 4-h debone time, the Pectoralis from 52-d-old broilers had greater ($P<0.05$) pH values than that of the 32-d-old broilers at all debone times. Previous research has indicated that larger birds with larger muscles will progress through rigor at a slower rate compared to smaller birds (Cooper and Fletcher, 1997; Berri et al., 2001; Brewer et al., 2012a), and this difference in rate of pH decline has been attributed lower glycolytic potential of larger muscles and slower PM glycolysis (Berri et al., 2001; Bihan-Duval et al., 2001). Results of the current study concur with previous research that older birds, with larger *Pectoralis* muscles, are progressing through rigor at a slower pace compared to younger birds with smaller *Pectoralis* muscles (Cooper and Fletcher, 1997; Berri et al., 2001). Interestingly, well beyond the completion of rigor (24 h PM), birds slaughtered at 52d of age had

greater (P<0.05) pH values than birds slaughtered at 32 d of age, perhaps indicating that decreased rate of glycolysis early PM is never overcome even as carcasses age until 24 h PM.

Texture is the most important attribute for consumer acceptance of meat products (Morgan et al., 1991), and it is traditionally recommended that breast fillets be aged on the bone 4 to 6 h to prevent toughness (Stewart et al., 1984; Lyon et al., 1985; Dawson et al., 1987). In the current study, both debone hour and bird age at slaughter affected texture of boneless breast fillets as indicated by differences in MORS force and energy data (Table 2). Shear properties (force and energy) for each debone hour generally decreased the longer fillets were left on the bone prior to deboning. For the 38-d-old birds, MORSE values declined (P<0.05) between 2 and 4, 3 and 6, and 4 and 8 h PM, but no (P<0.05) differences were noted between any other PM deboning hours. Conversely MORSE declined (P<0.05) only between 2 and 3 h PM in fillets form 52-d-old birds, and there was no (P<0.05) difference in MORSE among fillets deboned from 2 to 24 h PM. A similar trend was observed for MORSF values, with No (P<0.05) difference in MORSF at the 2- and 3-h debone times in the fillets of the 52-d-old birds, but the MORSF of the 24-h deboned fillets was less (P<0.05) than MORSF of fillets deboned at 6 h PM. These results indicate that holding fillets for just 1 hour after removal from the chill tanks to 3 h PM could produce a fillet texture similar to a 4-h PM, regardless of slaughter age. Also, results of this study would indicate that progression of rigor has a greater effect on breast fillet texture of birds reared to 32 d than birds reared to 52 d, because there was no significant decline in shear values between 3 and 24 h PM for the older birds.

Within each debone hour, shear values between ages were greater (P<0.05) at all debone hours for the 52-d compared to the 38-d-old birds. The increased shear force values for the 52-d-old birds could be attributed to the decreased rate of glycolysis PM, as evidenced by pH decline;

however, the decreased rate of pH decline would not account for the increased toughness at 8 and 24 h PM, well beyond the completion of rigor.

Cavitt et al. (2005) used MORS to predict consumer acceptance and preference of boneless breast fillet tenderness. Based on that study, the MORSE values in the current study would indicate that consumers would prefer the texture (in terms of degree of likeness) of the 38-d-old broilers at all debone times compared to the 52-d-old broilers. For example, considering the 2 h debone in the current study which resulted in the greatest shear values, fillets from the 38 d old broilers be classified as "like slightly" by consumers while fillets from 52 d old broilers would be classified as "dislike slightly" by consumers. Furthermore, fillets from the 32 d old broilers deboned at 3 h PM would be considered "moderately tender" and "liked moderately," and it is required that fillets from the 52 d old broilers be aged on the bone until 6 h PM to obtain a similar MORSE value and be classified as the same consumer acceptance category. Moreover, fillets deboned at 24 h PM form the 38 d old broilers would be classified in the "like extremely" and "extremely tender" category while the fillets from the 52 d old broilers would be classified in the "like moderately" category, no different than the 6 h deboned fillets of the 52 d old broilers.

Several studies have reported the increases in shear properties of breast fillets from birds at older ages and greater live weights at processing (Cooper and Fletcher, 1997; Poole et al., 1999; Northcutt et al., 2001). Poole et al. (1999) reported that broilers reared to 5 weeks of age were more tender than broilers reared to 6, 7, and 8 weeks of age. In that study, the 8 week old broilers had fillet weights of 538.3 g; in the current study the average fillet weight for the 52 d old birds was 598.2 g, potentially indicating a difference in growth potential of the birds used in this study compared to Poole et al. (1999). Northcutt et al. (2001) found, there was an effect of bird age on fillet texture when deboned at 0 and 2 h PM; however, there was no effect of age on

breast fillet texture when deboned later at 4 and 6 h PM. This would suggest an interactive effect of deboning time and age on fillet texture, where deboning early (pre-rigor) may impact texture differently than deboning later (post-rigor). Northcutt et al. (2001) also concluded there was no need to age broiler carcasses to 6 h PM, because they failed to note a change in shear force values between deboning at 4 and 6 h PM. Yet, in a more recent study, Brewer et al. (2012b) concluded that higher-yielding strains required aging to 6 h PM to reach equivalent tenderness with lower-yielding strains. Because 52-d-old broilers required deboning at 6 h PM to achieve shear force values equivalent to 38-d-old broilers deboned at 3 h PM, deboning time may need to be altered based on the commercial broiler strain to optimize meat tenderness.

In an attempt to understand the tenderness differences between the slaughter ages in this study, moisture and fiber diameter were measured. Moisture loss during cooking, as well as moisture content of cooked samples were used to measure WHC in the present study (Table 2). Regardless of deboning time, cook losses and cooked meat moisture did not differ ($P<0.05$) between slaughter ages. Only fillets from 52-d-old broilers deboned at 4 h PM had greater ($P<0.05$) cook losses when compared to those deboned at 0.25 h PM; however, this difference did not correspond with tenderness differences between these two debone hours. Thus, results of the present study indicate that reductions in moisture losses or total moisture content were not the cause of increased shear properties for birds reared to older ages.

Myofibrillar diameter was measured to assess muscle fiber properties of commercial broilers at 38 and 52 d of age, samples for fiber diameter were measured only on fillets deboned at 24 h PM to minimize the potential effect of debone hour on fiber diameter. Birds slaughtered at 52 d had greater ($P<0.05$) fiber diameters than birds slaughtered at 38 d of age (Table 3). This finding would indicate that muscle fiber hypertrophy was quite rapid during the 14 d between

slaughter ages. Moreover, this increased fiber diameter may be related to the late PM toughness observed in older commercial broilers. Previous research has indicated a linear relationship between increased fiber diameter and increased shear force values (Herring, 1930, Tuma et al., 1962; Crouse et al., 1991). However, all of these experiments were conducted with red meats, and there is very little evidence of this effect in poultry. So, the effect of rapid growth on the structure of the muscle, in particular total and soluble collagen contents, should be evaluated in future research.

Conclusion

The results of this study indicate that there is some factor in the modern commercial broiler when reared to older ages that are causing increased shear properties at later PM deboning times, and as pH values in this study indicate this difference is not related to rate of rigor development. In summary, birds reared to 32 d of age progressed through *rigor mortis* at a quicker pace than birds reared to 52 d of age, and the difference in PM pH decline associated with the progression of rigor was more positively correlated with MORSE of the 38-d-old birds ($r=0.36$) than with the 52-d-old broilers ($r=0.11$). Also, results of this study indicate that it would take fillets from 52-d-old broilers an additional 3 h of aging on the carcass (to 6 h PM) to reach MORSE values similar to 38-d-old broilers. Furthermore, shear force values of fillets from 38-d-old broilers continued to decrease until 6 h PM, whereas there was no decrease in MORSE values beyond 3 h PM for fillets from the 52-d-old broilers. Even when deboned well beyond the completion of rigor (24 h PM) fillets from 52-d-old birds are still tougher than fillets deboned from 38-d-old birds. It appears that much of this late PM toughness could be attributed to increased myofibrillar diameter of the older birds; yet, the effect of this growth on muscle, as well as and collagen content and solubility should be evaluated in future research.

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Table 1: Age main effect on growth and fillet dimension parameters

	Bird age[1]		Difference	Pooled
	38 d	**52 d**	**%[2]**	**SEM**
Live weight, g	1644[b]	3145[a]	91.3	48.08
RTC[3] yield %	72.0[b]	77.6[a]	7.8	0.30
Fillet weight, g	115.2[b]	299.1[a]	159.5	5.39
Fillet yield %[4]	10.3[b]	11.8[a]	14.6	0.06
Fillet length, cm	12.3[b]	15.6[a]	27.4	0.12
Fillet width, cm	6.26[b]	8.18[a]	30.7	0.07
Cranial thickness, cm	2.32[b]	3.58[a]	54.3	0.04
Midpoint thickness, cm	1.40[b]	2.16[a]	54.3	0.03
Caudal thickness, cm	1.98[b]	2.97[a]	50.0	0.04

[a-b] means within column for one parameter without common superscript are significantly different ($P<0.05$)

[1]n=48 per mean

[2]Difference in dimension calculated as a percent of the 38 d fillet dimension

[3]Ready-to-cook

[4]Single fillet yield calculated as percent of RTC weight

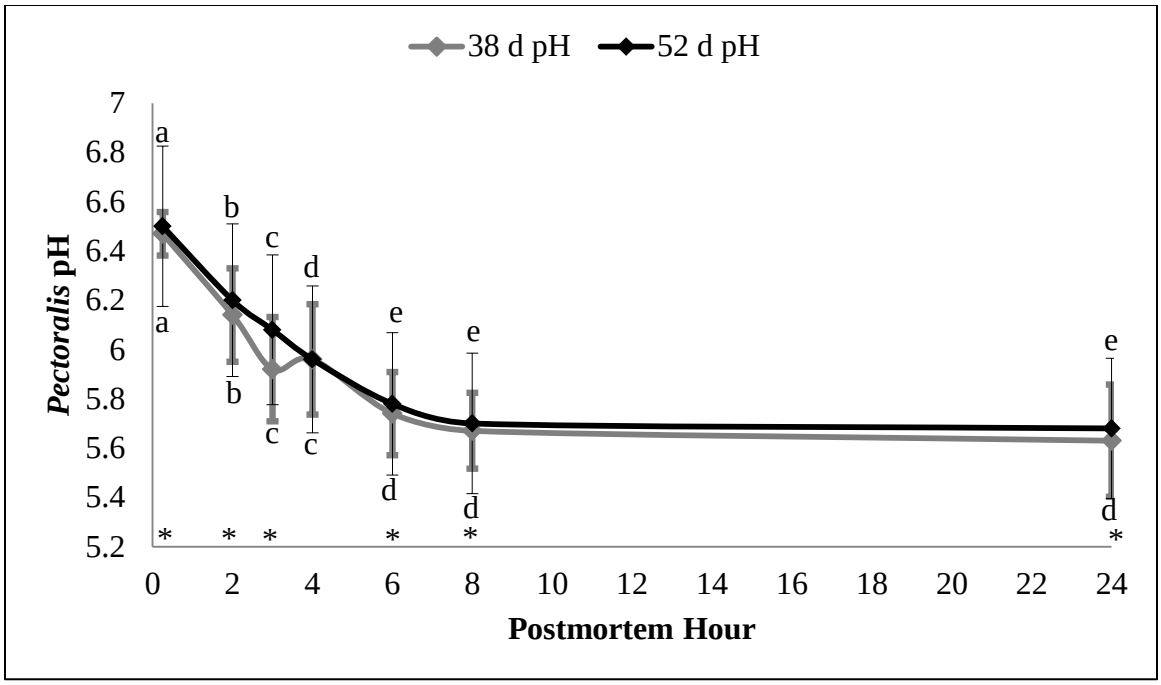

Figure 1: *Pectoralis* pH of 38 and 52 d old broilers at variable debone times. a-e Means among debone hour without common letter differ significantly ($P<0.05$). *Indicates significant difference ($P<0.05$) at postmortem hour between slaughter ages. Pooled SEM: 38 d old broilers=3.50; 52 d old broilers=2.97. n=24 per mean

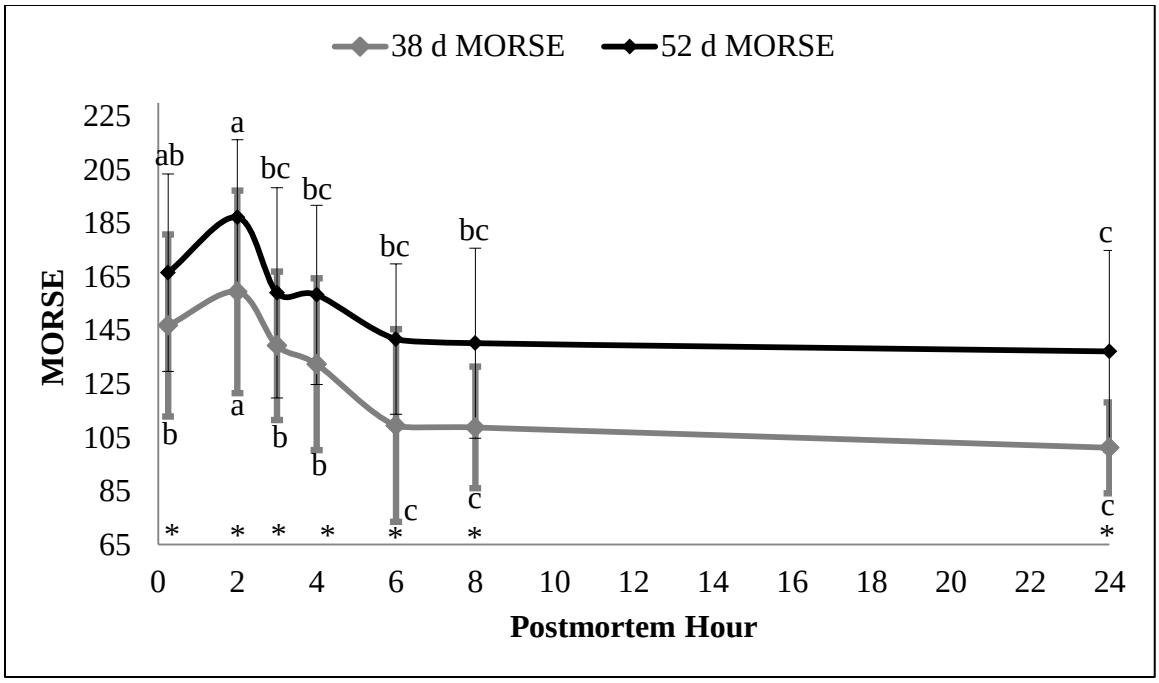

Figure 2: MORSE for 38 and 52 d old broilers at variable debone times. a-e Means among debone hour without common letter differ significantly ($P<0.05$). *Indicates significant difference ($P<0.05$) at postmortem hour between slaughter ages. Pooled SEM: 38 d old broilers = 3.50; 52 d old broilers = 2.97. n=24 per mean

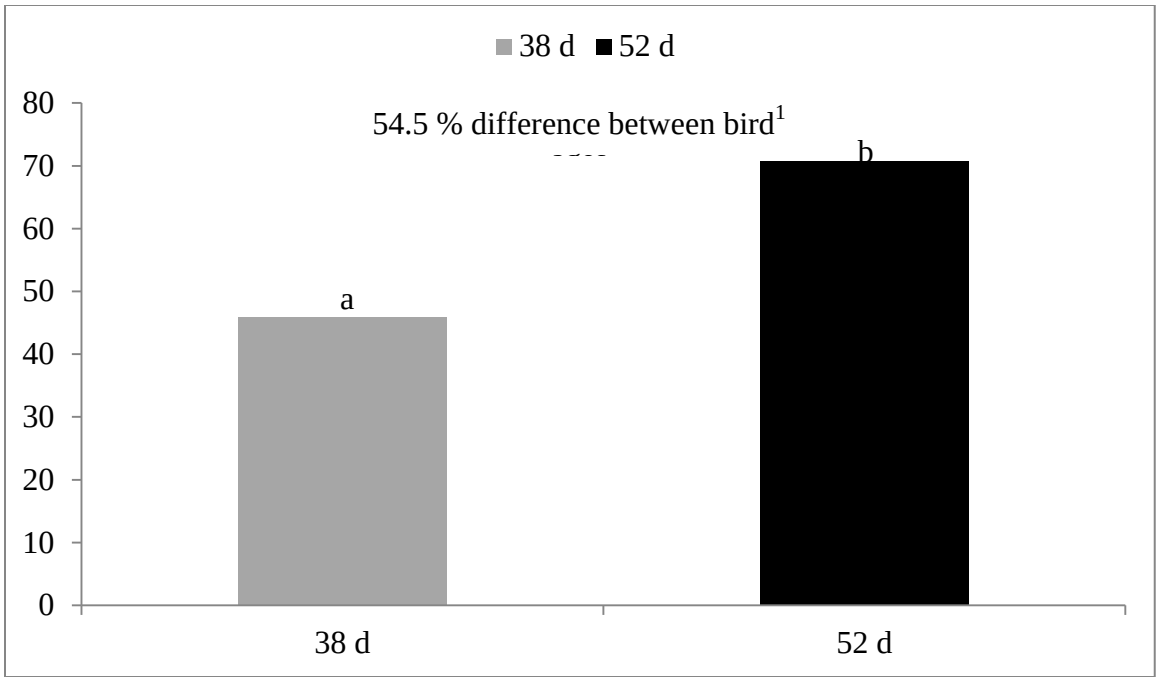

Figure 3: Myofibrillar diameter (µm) of breast fillets from small and large broilers deboned at 24 h PM. a-b means without common superscript are significantly different ($P<0.05$). 1Difference in diameter calculated as a percent of the 38 d fiber diameter. Pooled SEM: 38 d old boilers = 41.9; 52 d old broilers 21.2. n=24 per mean

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December 2, 2013

To whom it may concern:

This certifies that Valerie Brewer serves as the first author on the paper, "Tenderness of broilers reared for divergent market demands and deboned at varying times pre- and post-rigor." She completed over 51% of the associated research/work for the paper.

Casey M. Owens, Ph.D.
Major Professor and Co-author

TO: Casey Owens-Hanning
FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee
DATE: August 23, 2011
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: August 21, 2014

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12004- "PHYSICAL AND BIOCHEMICAL PROPERTIES ASSOCIATED WITH TENDERNESS IN BIG AND SMALL BIRD MARKET PROGRAMS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-14-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

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Chapter 4

Tenderness of non-marinated and marinated commercial broiler breast fillets from birds reared to 60 d

V.B. Brewer and C.M. Owens

Abstract

Commercial broilers are commonly reared to around 8 weeks of age to meet the market demands for boneless breast meat. Research has indicated that birds reared to this age could potentially be tougher than younger broilers, and marination is commonly used to alleviate the toughness associated with early deboning of these older birds. Therefore, a study was conducted to evaluate the effect of debone hour and marination on the texture of breast fillets from broilers reared to 60 d of age. A total of 210 male broilers were slaughtered and deboned at 0.25, 2, 3, 4, 6, 8, or 24 h PM in two replicates. Samples were collected for pH analysis from all breast fillets. One fillet from each broiler was tumble marinated (0.75% NaCl and 0.45% phosphate, final concentration). Fillets were cooked on raised wire racks in covered pans, and shear force analysis was measured using Meullenet Owens razor shear (MORS) method on all fillets. There was a decline ($P<0.05$) in pH at all debone hours except between 8 and 24 h PM. Marination uptake and cook loss were evaluated, neither was affected by debone time ($P<0.05$). The 2 h PM deboned fillets had the greatest ($P<0.05$) MORS energy, but the only significant decrease ($P<0.05$) in MORS energy was between the 2 and 4 h PM debone times. From 3 to 24 h PM, MORS energy did not change ($P<0.05$) among non-marinated fillets. Even at 24 h PM, the non-marinated fillets had unacceptably high MORS energy values; however, marination improved breast fillet tenderness at all debone hours as indicated by lower ($P<0.05$) MORS energy in marinated compared to non-marinated fillets. Among marinated fillets, there were differences ($P<0.05$) in tenderness between 0.25 and 2, and 3 and 4 h PM. Results of this experiment would indicate that toughness could be an issue for non-marinated fillets from large broilers even when deboned at 24h PM. However, tumble marination can alleviate the toughening effect associated

with large broilers, even though shear force values of marinated fillets can still be affected by deboning time.

Key Words: broiler breast fillet, early deboning, marination, shear energy

Introduction

In the commercial broiler industry, it is not uncommon for broilers to be reared to a live weight of 8 pounds, or heavier live weight to meet consumer demand for boneless breast meat and further processed products. Processing large broilers with high breast-meat yield allows for greater pounds per man hour to be produced, thereby leading to improved processing efficiency. Processing efficiency can be further improved through deboning carcasses immediately upon leaving the chill tank, resulting in streamlined poultry processing, reduced storage space for aging carcasses, and improved boneless meat yields when deboned early (Hirschler and Sams, 1998). However, in commercial processing, the carcasses will be approximately 1.75 to 2 h postmortem (PM) when chilling is complete, and increased shear force values, as well as, decreased consumer satisfaction, are commonly associated with breast meat deboned at this time PM (Stewart et al., 1984; Lyon et al., 1985; Dawson et al., 1987; Cavitt et al., 2005; Xiong et al., 2006).

Previous research evaluating broiler breast fillet tenderness and the MORS texture analysis method (Cavitt et al., 2004; 2005) indicated that the maximum shear energy for broiler breast fillets would be approximately 172 N.mm. However, recent research has indicated that shear energy values, using the MORS method, of currently available commercial broiler strains reared to 8 lb. live weight could potentially be greater than this (Brewer et al., 2012b). In two separated studies evaluating the effect of broiler strain on meat quality of birds reared for small and big bird market programs, Brewer et al. (2012a, b) reported MORS energy values of 144 to

163 N.mm when deboned at 2 h PM, whereas birds reared to 60 d of age produced MORS energy values between 187 and 192 N.mm and both results were dependent upon strain. Using the MORS method, Cavitt et al. (2005) concluded that a consumer would find shear force values of the small birds to be more acceptable than shear force values from the big birds. Differences between these studies were likely due to bird age and, therefore, size at slaughter. Fortunately, these larger broilers often are used for further processed products and will often be portioned and marinated during processing. Marination will affect not only flavor, but also tenderness and water-holding capacity; therefore, marination often serves as an intervention for breast fillet toughness associated with pre-rigor deboning (Saha et al., 2009; Lopez et al., 2012),

It is estimated that greater than 50% of the commercial broiler market will undergo some marination step during processing (Smith and Acton, 2010). Common commercial marinades will include functional ingredients, such as salt (NaCl) and phosphate, and previous research has indicated that NaCl concentrations of as little as 0.5% can improve sensory attributes of boneless breast fillets (Saha et al., 2009; Lopez et al., 2012). This improvement in consumer acceptance is the result of improving tenderness through solubilization of myofibrillar proteins, and by improving the juiciness by increasing interstitial space for moisture to be held (Hamm, 1972; Alvarado and Sams, 2003; Gorsuch and Alvarado, 2010; Woelfel and Sams, 2001).

Marination can be applied to boneless breast fillets via injection or vacuum tumbling or a combination of the two methods; however, vacuum tumbling is used most commonly in the commercial broiler industry (Smith and Acton, 2010). There have been mixed reports on the efficacy of vacuum tumbling (Young and Lyon, 1997; Young et al., 2004; Smith and Young, 2007); however, there is consensus among previous research that it is the tumbling action not necessarily the decrease in atmospheric pressure, which will improve marinade uptake when

compared a static marination of breast fillets (Chen, 1982; Heath and Owens, 1991; Young and Smith, 2004). Yet, Alvarado and Sams (2004) determined that early PM (3 h) injection and tumbling of boneless breast fillets resulted in increased toughness of boneless breast fillets, as well as uneven distribution of sodium ions throughout the breast fillets. Because of the potential uneven marinade distribution, it could be assumed that tumble marinating alone may not be sufficient for improving the tenderness of breast fillets harvested from birds reared to heavier live weights, which can have fillet weights in excess of 2.5 pounds (Brewer et al., 2012). Furthermore, when deboning early PM, contracted sarcomeres result in decreased interstitial space for moisture, and perhaps marinade, to be held; therefore, debone hour could potentially affect marination efficacy. Considering all of these factors, the purpose of this research was to evaluate the effect of vacuum-tumble marinating on boneless breast fillets from birds reared to 60 d of age and deboned at variable times PM.

Materials and Methods

Commercial broilers from a standard-yielding strain were reared at the University of Arkansas Broiler Research Farm in floor pens with feed formulated to meet all NRC (1997) requirements, and broilers had *ad libitum* access to feed and water. Feed was withdrawn 8 to 12 hours before birds were cooped and transported to the University of Arkansas Pilot Processing plant, where they were commercially processed on an inline system (Mehaffey et al., 2006; Brewer et al., 2012). Birds (n=210) were randomly assigned to debone hour treatment (n=30 per treatment), and processed in two replications. Following processing, birds were chilled in ice water baths, which included a pre-chill (0.25 h at 13^{o}C) and chill (1.75 h at 1^{o}C), excluding one treatment which was deboned immediately post-evisceration (0.25 h postmortem). After chilling, carcasses were well packed and aged on ice until the appropriate debone time at 3, 4, 6,

8, or 24 h postmortem (PM), as well as an additional deboning treatment immediately post-chill (2 h PM).

Breast fillet deboning was completed by 3 trained and experienced people. Following deboning the caudal tip of the left breast fillet was removed for pH evaluation, and the right breast fillet was weighed for determining fillet yield. Samples for pH evaluation were immediately frozen in liquid nitrogen, and pH was measured using the iodoacetate procedure of Sams and Janky (1986) to monitor rigor progression. The remainder of the fillet was packaged in zip-sealed bags and stored overnight at 4C. At 24 h PM, the last treatment was deboned and all of the fillets were frozen for later determination of cook loss and shear properties.

Fillets were removed from the freezer 48 h prior to cooking to ensure they were adequately thawed. Left breast fillets were tagged for identification and then tumble marinated in a brine solution with 0.75% NaCl and 0.45% phosphate (Carnal 822; Budenheim USA Inc., Plainview, NY) for 25 minutes, with a target marination uptake of 15% of pre-marinated fillet weight. Marination was completed in 10 replicates, with fillets from each debone treatment randomly assigned to each marination replicate. Following vacuum tumbling, fillets were allowed to rest 10 minutes prior to weighing for calculating marination uptake. Both left and right breast fillets were cooked in aluminum foil covered pans on raised wire racks in a convection oven to an internal temperature of 76^{o}C (Cavitt et al., 2004; Mehaffey et al., 2006; Brewer et al., 2012). A scanning thermometer (Digi-Sense Scanning Thermometer, Model # 69200-00, Eutech Instruments Pte Ltd, Singapore, China) was used to monitor internal temperature throughout the cooking process. Following cooking, fillets were cooled and weighed for calculating cook loss percentage. Fillets were then individually wrapped and stored overnight at 4^{o}C for texture analysis. Shear properties were determined using the Meullenet-

Owens Razor Shear Method on a Texture Analyzer Model TA-XT2i (Texture Technologies, Scarsdale, NY) with three replicate shears taken per fillet. The razor blade was changed after 30 fillets to ensure that the blade did not dull.

Data was analyzed using the GLM procedure (SAS, 2010). Main effects in this study were postmortem deboning time and marination treatment, whereas debone replication was included in the model as a random variable. There was no effect ($P<0.05$) of marination replication on marination pickup or shear properties; therefore, it was excluded from further analysis. Means were separated using Duncan's Multiple Range test and significance was considered at $P<0.05$.

Results and Discussion

Average live weight for the birds used in this study was 3.5 kg (Table 1), which is not an uncommon market weight to meet the growing demands for further processed poultry products. Single fillet yield in this study was 12% of ready-to-cook weight (average fillet weight 331 g), which is comparable to fillet yield for this age of broiler (Brewer et al., 2012; Kuttappan et al., 2012).

Postmortem pH decline of the breast muscle was measured in this study to monitor progression of rigor at the variable debones times (Figure 1). *Pectoralis* pH is expected to decline due to the buildup of lactic acid from PM anaerobic metabolism in the muscle. In the current study, *Pectoralis* pH decreased ($P<0.05$) at each time point between 0.25 and 8 h PM, but pH did not change ($P<0.05$) between 8 and 24 h PM. In contrast, Kiojowski et al. (1982) determined that *Pectoralis* pH declined between 30 min and 1 h PM and between 1 and 2 h PM, but PM pH did not change at any other PM time evaluated. In that particular study, carcasses were held without chilling prior to debone, which would have accelerated the progression of

rigor development. Stewart et al. (1984) and Lyon et al. (1985) found no differences in pH decline after 4 h PM in broiler breast fillets deboned at variable times when carcasses had been immersion chilled. It should be noted that several generations of selection for breast meat yield has occurred between the broiler strains used in the previously mentioned studies and current broiler genetic strains. Furthermore, research has also demonstrated that broilers with greater body and fillet weights will have a reduced rate of pH decline, as a result of lower glycolytic potential of the breast fillet, and decreased rate of PM glycolysis (Cooper and Fletcher, 1997; Berri et al., 2001). More recent research has indicated that pH, particularly in large high-yielding birds, will decline beyond 4 h PM (Brewer et al., 2012) which is in agreement with results of the current study.

Fillets were cooked prior to shear analysis and cook loss was determined to evaluate water-holding capacity because of the significant impact that it can have on tenderness. In this study, debone hour did not affect ($P>0.05$) cook losses of non-marinated or marinated fillets (results not presented). However, non-marinated fillets had greater ($P<0.05$) cook loss than marinated fillets (29.9 vs. 28.5%, results not presented). Research has indicated that debone hour can affect cook loss, which is typically attributed to decreased interstitial space for water binding, however, there is not always a clear relationship between debone time and cooking loss percentage (Mehaffey et al., 2006; Brewer et al., 2012).

Tenderness is an important meat quality attribute and can be affected by various factors, including broiler genetic stain, bird age, PM deboning time, and marination (Poole et al., 1999; Northcutt et al., 2001; Cavitt et al., 2004; Mehaffey et al., 2006; Saha et al., 2009; Brewer et al., 2012). Shear force values (MORS force and energy) generally declined ($P<0.05$) as deboning time increased (Table 1). For non-marinated fillets shear energy was greatest ($P<0.05$) when

fillets were deboned at 2 h PM (245.5 N.mm), and fillets deboned at 4 h PM, or after, had lower (P<0.05) MORS force and MORS energy compared to those deboned at 0.25 and 2 h PM. Regardless of decreasing MORS energy over deboning times, MORS energy for non-marinated fillets at all PM debone hours would be rated by consumers as "disliked very much" and "extremely tough" (Cavitt et al., 2005; Xiong et al., 2006). Even though MORS energy did not (P<0.05) Fillets deboned at 8 and 24 h PM which did not differ (P>0.05) would still be considered "very tough" and "disliked moderately" by consumers based on the results of Cavitt et al. (2005).

One benefit of marination is that it can alleviate the toughening effect associated with early PM deboning of broiler breast meat. Marination properties can be affected by various factors, such as debone time, marination time (prior to and duration), and functionality of proteins (Alvarado and Sams, 2004; Alvarado and McKee, 2007; Xiong and Brekke, 1999). Researchers have previously reported that debone hour could potentially affect marination uptake because of less interstitial space for bound water to be held in the muscle (pre-rigor deboning), as well as decreased functionality of proteins in post-rigor deboned meat (Alvarado and McKee, 2007; Xiong and Brekke, 1999). Even though the, average marination uptake (11.3%), was lower than the target of 15%, marination uptake was similar (P<0.05) among all deboning times (results not presented). Researchers have reported that marination uptake and efficacy can be affected by debone time (Alvarado and Sams, 2004; Kuttappan et al., 2010). Additionally, research has shown when fillets were marinated pre-rigor, when deboned pre-rigor, had less marination pickup (Kuttappan et al., 2010). However, in the current study, fillets were not marinated immediately post deboning therefore, debone hour did not affect marination uptake in this study.

Tumble marinating fillets decreased ($P<0.05$) fillet shear values at all debone times when compared to non-marinated fillets (Figure 1). Also, shear values for marinated and non-marinated fillets were positively correlated (r=0.76 and 0.81 for MORSF and MORSE, respectively, $P<0.0001$), indicating that, shear values generally decreased in the marinated fillets the longer the meat was left on the bone prior to deboning. As with the non-marinated fillets, 2 h shear values were greater ($P<0.05$) than shear values from fillets deboned at 4 h PM, and after, and similar ($P<0.05$) to shear values of fillets deboned 3 h PM. Alvarado and Sams (2004) reported that tumble marinating immediately post-debone can cause increased shear force values; however, this was not observed in the current study because marinating did not occur immediately post debone. For the current study, MORS energy values of the marinated fillets deboned at 2 and 3 would equate to consumer evaluations of "neither disliked nor liked" for tenderness acceptability and "moderately tough" for tenderness intensity, respectively an improvement over the ratings of "disliked very much" and "extremely tough" for the non-marinated fillets (Cavitt et al., 2005). Lowest MORS ($P<0.05$) energy values were for marinated fillets deboned at 4 and 24 h (129.6 and 126.5 respectively) and shear values in this range would equate to "like moderately" and "moderately tender" category for tenderness acceptance and intensity respectively (Cavitt et al., 2005).

In the current study, 0.75% salt concentration in the total formula was used for marinating fillets because Saha et al. (2009a) concluded that this was the most appropriate level to yield products with the greatest percentage of consumer acceptability. In addition, 0.75% salt concentration resulted in average shear energies of 103.8 N.mm when fillets were deboned at 5 h PM (Saha et al., 2009). In the current study, marinated fillets had MORS energies that were greater than this at all PM debone hours. It should be noted that Saha et al. (2009a) slaughtered

commercial broilers at 42 d of age, and the differences in shear values between that study and the current study can be attributed to differences in bird age at slaughter. Moreover, even though birds were slaughtered at 56 d of age in Saha et al. (2009b), very few differences in texture were noted by consumers between pre- and post-rigor deboned breast fillets marinated in a solution containing 1% salt. These findings were in contrast to others (Lyon et al., 1985; Cavitt et al., 2005; Xiong et al., 2006) who demonstrated that consumers could detect differences between pre- and post-rigor deboned fillets, with fillets deboned pre-rigor receiving lower consumer acceptability of boneless breast fillets. Saha et al. (2009b) attributed the lack of differences found to the level of salt used in the marinade. A salt concentration of 1% was used and although it resulted in no significant difference found in samples deboned pre and post rigor, 20.5 % of consumers found the salt concentration of 1% to be "too salty."

Fillet thickness at the cranial portion was measured in the current experiment because research has suggested that fillet that weigh more and are thicker fillets will have higher shear values (Lubritz, 1997; Brewer et al., 2012). This could be due to sarcomere shortening and/or muscle size (e.g., fiber diameter). In the current study, fillet height at the cranial region and fillet yield were positively correlated ($r=0.68$, $P<0.0001$); however, there was not a strong relationship between fillet thickness and shear values for the marinated or non-marinated fillets. This lack of difference in the current study may be attributed to the limited range of bird size (weight), and the general lack of difference in shear values between debone hours. In contrast, Brewer et al. (2012) compared several standard and high yielding commercial strains resulting in a larger range in bird size (weight), and found that strains with thicker fillets also had greater shear values. The results of this study would imply a need to elicit the relationship, and possible predictive value of fillet thickness to shear properties in future research.

Conclusion

In summary, results of the current study indicate, tumble marination alone will effectively reduce shear values of boneless breast fillets from large broilers, even though marination penetration was not a factor. Although marination produced the lowest shear values, these values would still be considered only "moderately tender" by consumers. Moreover, increased shear values of these large fillets may be attributed to decreased rate of rigor development as indicated by muscle pH values, which declined significantly up to 8 h PM. At these pH values, it is plausible that there could still be energy in the muscle to cause contraction of the sarcomere later PM than reported previously. Muscle pH declined at each debone hour until 8 h PM but, there was not a significant decrease in MORS energy values after 3 h PM, and the relationship between pH decline and shear energy values (r =0.187) was not strong. These results would suggest that there is some other factor contributing to increased shear force in fillets from larger birds, apart from the effect of decreased rigor development.

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Table 1. Growth parameters of breast fillets from large broilers deboned at variable debone times.

Attribute	Trt[1]	Debone Hour (h) 0.25	2	3	4	6	8	24	Pooled SEM
Live Weight (g)		3382^{c}	3621^{ab}	3697^{a}	3296^{c}	3441^{bc}	3748^{a}	3393^{c}	22.69
Fillet Weight (g)		305.2^{c}	355.9^{a}	362.3^{a}	309.8^{c}	322.8^{bc}	350.3^{ab}	315.0^{c}	4.05
Fillet Yield		11.8^{c}	12.3^{ab}	12.4^{a}	11.8^{c}	11.9^{bc}	11.8^{c}	11.7^{c}	0.07
Cooked Fillet Cranial Height (mm)	NM	37.3^{ab}	38.6^{a}	38.9^{a}	35.8^{bc}	34.9^{c}	36.2^{bc}	35.1^{c}	0.28
	M	37.1^{ab}	38.2^{a}	37.3^{a}	33.4^{c}	36.6^{ab}	36.9^{ab}	34.8^{ab}	0.30
		NS	NS	NS	NS	NS	NS	NS	

[a-d] Means within row with no common superscript differ ($P<0.05$)
NS Means within column within attribute do not differ (P>0.05), non-significant
[1]Treatments: Non-marinated or Marinated
n= 30 per mean

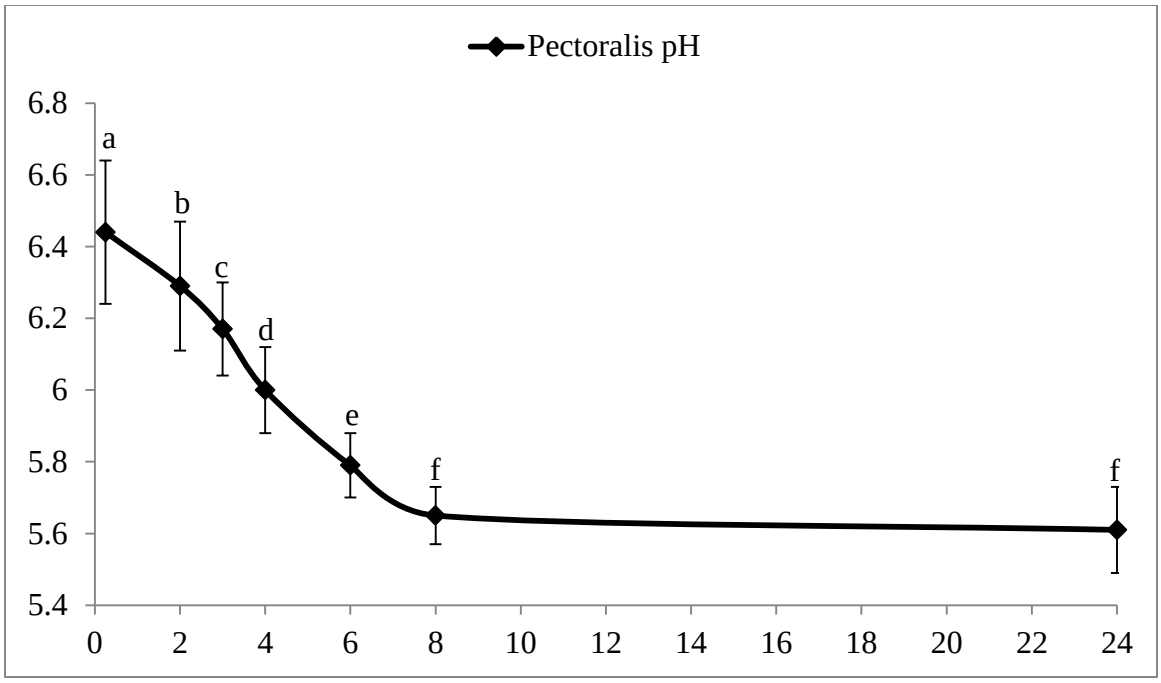

Figure 1.*Pectoralis* pH of large broilers at variable debones times. a-f Means with differing script are significantly different ($P<0.05$; Pooled SEM 0.02). n=30/mean

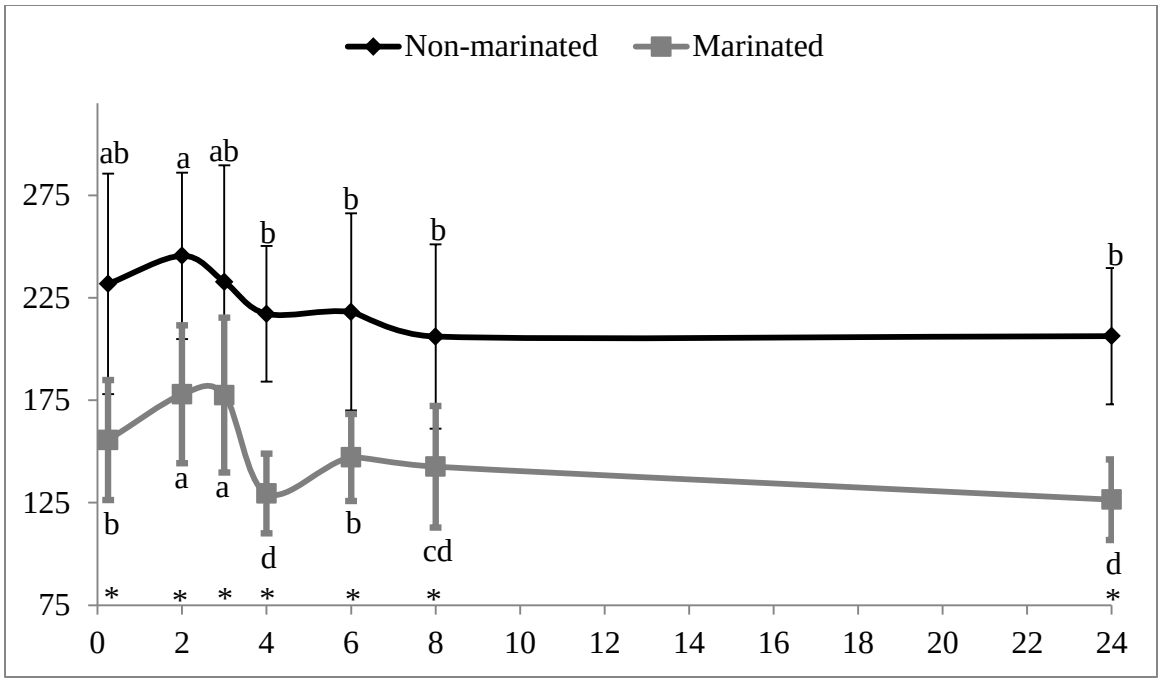

Figure 2: MORSE of non-marinated and marinated fillets from big birds at variable debone times. a-d Means within marination treatment without common script are significantly different ($P<0.05$). *Means within debone hour are between marination treatment are significantly different. Pooled SEM: Non-marinated=2.97; Marinated=3.50. n=30/mean.

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December 2, 2013

To whom it may concern:

This certifies that Valerie Brewer serves as the first author on the paper, "Tenderness of non-marinated and marinated commercial broiler breast fillets from birds reared to 60 d of age." She completed over 51% of the associated research/work for the paper.

Casey M. Owens, Ph.D.
Major Professor and Co-author

TO: Casey Owens-Hanning
FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee
DATE: August 23, 2011
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: August 21, 2014

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12004- "PHYSICAL AND BIOCHEMICAL PROPERTIES ASSOCIATED WITH TENDERNESS IN BIG AND SMALL BIRD MARKET PROGRAMS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-14-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Chapter 5

Physical and biochemical properties effecting breast fillet tenderness of commercial and random bred strain broilers reared to 55 d

V.B. Brewer and C.M. Owens

Abstract

Toughness of young broilers has primarily been attributed to contractile toughness resulting from early deboning and sarcomere shortening. However, increased fillet toughness in large market broilers has been observed regardless of deboning time. Therefore, this study evaluated biochemical and physical characteristics of broiler meat from modern commercial (Strain A and B) and random bred (Strain C; White Plymouth Rock × Hampshire) broilers grown to 55 d. Broilers (n=180) were processed over 3 replications, and carcasses were deboned at either 2 or 6 h postmortem (PM). Muscle pH, fiber diameter, sarcomere length, cook loss and Meullenet-Owens razor shear were analyzed on breast fillets. Broilers of Strain A were heaviest (P<0.05), and broilers from Strain C were the lightest (P<0.05), at slaughter. As expected, Strains A and B (the commercial strains) produced greater fillet yields than Strain C. Muscle pH decreased (P<0.05) between 2 and 6 h PM in fillets from Strains A and B. Conversely, although pH values were similar (P<0.05) between 2 and 6 h PM in fillets from Strain C, fillet pH was less at 2 h PM than both Strains A and B. Regardless of strain, cooking losses were greater (P<0.05) when deboned at 2 than 6 h, and cooking loss was greater (P<0.05) in Strain B than C at 2 h PM, as well as greater (P<0.05) in Strain A than Strain C at 6 h PM. Fillets deboned at 2 h had greater (P<0.05) shear force values than those deboned at 6 h, whereas fillets from Strain B had greater (P<0.05) shear force compared to Strain A fillets when deboned at 2h PM, and fillets from both Strain A and B had greater (P<0.05) shear force than Strain C when deboned at either 2 or 6h PM. Strain C fillets had longer (P<0.05) sarcomeres than Strains A and B at 2 h PM, but Strain B fillets had greater (P<0.05) sarcomere lengths than Strain C at 6 h PM. Even though PM proteolysis (as measured by gravimetric fragmentation index) was similar (P<0.05) among

strains, fiber diameters were greater ($P<0.05$) in fillets from Strains A and B than those from Strain C. These results suggest that factors other than sarcomere shortening are involved in toughness in modern, large broilers.

Introduction

Commercial broilers have been selected for generations for improved meat yield, specifically breast fillet yield, because of the increased demand for breast meat and further processed products. To meet demand, as well as improve efficiency and profitability, several strains are available which have greater breast yields than other commercial strains (Biligili et al., 1991; Acar et al., 1991; Brewer et al., 2012).

There is some indication that broiler strain can affect meat quality (Mehaffey et al., 2006; Lopez et al., 2011; Brewer et al., 2012b,c). Brewer et al. (2012b, c) found that shear force values were increased in high-yielding strain, especially when deboned at 2 and 4 h PM, particularly when deboned early postmortem (PM). Traditionally, toughness in broiler breast fillets is attributed to contraction and shortening of the sarcomere when deboning occurs pre-rigor. Moreover, research has indicated that high-yielding strains progress though rigor mortis at a slower rate than birds than lower yielding strains (Berri et al., 2001). This finding could indicate that high-yielding birds could require longer PM aging times to prevent sarcomere contraction upon deboning, and to reach comparable tenderness values to lower yielding strains.

Several studies have been conducted to evaluate the effect of strain on breast fillet yield, histology, and meat quality. Breast fillet thickness at the cranial end has been correlated with fillet yield in several studies (Lubritz, 1997, Scheuermann et al., 2003, Brewer et al 2012). Scheuermann et al. (2003) found no difference in fiber diameter between commercial strains measured at 9 ages between 1 and 57 d of age, but attributed differences in yield to myofibrillar

number. However, it is commonly accepted that post-hatch muscle grows primarily through hypertrophy of the muscle fiber, and, in red meat, increased muscle fiber diameter is associated with increase shear force values (Morgan et al., 1991; Smith 1963). Therefore, the effect of myofibrillar diameter of strains should be evaluated for its effect on shear properties.

Postmortem proteolysis occurs during the resolution of rigor. It has been hypothesized that proteolysis in broilers weakens the myofibrillar lattice (McKee et al., 1997), whereas, in red meats, proteolysis degrades primarily the Z-disc and desmin, resulting in extensive postmortem tenderization (Shackelford et al., 1994; Koohmaraie, 1994). Although the exact activity of proteolysis is debated between red meat and poultry, it still plays a role in PM meat tenderness, and the effect of proteolysis on meat tenderness between high-yielding and standard-yielding commercial broiler strains has not been evaluated to date.

To evaluate the effect of strain on boneless breast fillet tenderness, birds of 3 strains were obtained for this study. Thus, the effect of selection for growth, breast meat yield, and meat quality in particular factors that could affect tenderness of boneless breast fillets were evaluated among the strains at deboning times of 2 h (pre-rigor state) and 6 h (post-rigor state) PM.

Materials and Methods

Strains used in this experiment included a standard-yielding commercial strain, a high-yielding commercial strain and a random bred strain (White Plymouth Rock × New Hampshire from 50 years of natural mating). Male broilers were reared to 55 d of age in floor pens on pine wood shavings, with *ad libitum* access to feed and water. Feed, which met all NRC requirements, was withdrawn 10 h prior to processing before birds were cooped and transported to the University of Arkansas Pilot Processing Plant. In 6 replicates birds were commercially processed by low-voltage stunning, scalding, picking, and evisceration on an in-line system as

described by Mehaffey et al. (2006). After evisceration, carcasses were chilled in agitated ice water baths. Chilling consisted a 0.25-h pre-chill at 13^{o}C, and a 1.25-h chill at 4^{o}C. After chilling, carcasses where either deboned at 2 h PM or well-packed and aged on ice until 6 h PM. Deboning was completed by 4 trained and experienced individuals. After debone, samples for pH were immediately cut and frozen in liquid nitrogen from the caudal portion of the right breast fillet, and then stored at -80C until analysis. The left breast fillet was weighed for yield calculation, and subsequently packaged in zip-sealed bag, packed on ice, and stored overnight in a 4^{o}C cooler. At 24 h PM, fillets were removed from cooler and samples were cut from the left breast fillet for sarcomere length (SL), gravimetric fragmentation index (GFI), and myofibrillar diameter (MD). Right breast fillets were vacuum packaged and frozen -80C for later determination of cook loss and shear properties.

Prior to cooking, fillets were removed from the freezer and allowed to thaw. Fillets were weighed and then cooked on raised wire racks in aluminum foil covered pans to an internal temperature of 76^{o}C. Internal temperature was tracked throughout cooking with a scanning thermometer (Digi-Sense Scanning Thermometer, Model # 69200-00; Eutech Instruments Pte Ltd, Singapore, China) to ensure proper end point temperature and prevent over-cooking. After cooling, fillets were weighed and individually packaged for shear analysis using the Meullenet-Owens razor shear (MORS) method on a TA-XT2i (Texture Technologies, Scarsdale, NY) analyzer. Replicate values (n=4/fillet) were averaged for fillet shear force (MORSF) and shear energy (MORSE) (Cavitt et al., 2005; Mehaffey et al., 2006).

Fillet pH was determined using the iodoacetate method described by Sams and Janky (1986). Briefly, frozen samples were pulverized and homogenized in an iodoacetate solution, and pH of the homogenate was measured using a Corning pH meter (Model 430; Corning

Industries, Corning, NY). Gravimetric fragmentation index was used to determine myofibrillar fragmentation according to the method described by Sams et al. (1991). Briefly, muscle samples were homogenized in an iodoacetate solution, vacuum filtered through a 250-µm nylon screen, and the residues were dried and weighed. When measuring myofibrillar diameter, samples taken from the right breast fillet deboned at 2 h PM were cut (approximately 0.5 × 0.5 × 1 cm) and fixed in formalin prior to staining. Then 2 replicate samples were fixed in paraffin blocks, cut on a microtome, and stained with hematoxylin and eosin. Diameters of muscle fiber (n=5/fiber) were measured on 8 fibers from each fixed and stained block. Using an Olympus microscope (Model Bx50; Olympus Corporation of the Americas, Center Valley, Pennsylvania), Optronix engineering scanner (Model DEI-470) and Image Pro Plus analysis software (Media Cybernetics, Inc., Warrendale, PA). All measurements were taken using 20 x magnifications. Sarcomere length was measured (8 diffraction patterns/sample) using the laser diffraction method described by Voyle (1971), as modified by Cross et al., (1980).

Data were analyzed as a random complete block design using the GLM procedure of SAS (SAS, 2002), with treatments in a 3 × 2 factorial arrangement. Main effects in the model included strain and debone hour along with the interactive effect, whereas slaughter replicate was included in the model as a random effect. When main or interactive effect was significant ($P \leq 0.05$), least squares means were separated using Duncan's multiple range test.

Results and Discussion

Live weight, RTC weight and fillet weight and yield were determined to illustrate the effect of the selection pressure applied to the high-yielding birds compared to the standard strain and unselected random bred strain (Table 1). In this study, there was a significant difference in live weight among all strains ($P<0.05$). The standard strain had a greater ($P<0.05$) live and RTC

weight than the high-yielding strain, and random bred strain was smaller ($P<0.05$) than the commercial strains. On the other hand, the high-yielding strain had greater yield than the standard-yielding strain ($P<0.05$), and both commercial strains yielded more than the random bred strain. As with RTC yield, the high-yielding strain had greater ($P<0.05$) fillet yield when deboned at 2 and 6 h PM than the standard-yielding strain despite the lower live weight of the high-yielding birds. For the random bred strain, as expected, fillet yield was dramatically less compared to both commercial strains ($P<0.05$). Deboning fillets at 6 h PM also resulted in lower ($P<0.05$) fillet yield for the standard and random bred strain compared to deboning at 2 h PM. Interestingly, for the high-yielding strain yield was similar ($P<0.05$) when fillets were deboned at 2 or 6 h PM. The observed decrease in fillet yield between deboning times is typical, and related to increased PM muscle proteolysis and muscle tearing associated with later PM debone times (Sams, 1999). It would appear that the high-yielding strains would result in greater profitability because they produced greater breast yields that changed little between deboning times compared to the standard-yielding strain (0.4 vs. 1.0% loss between deboning times)

Research has repeatedly shown that debone hour and strain can synergistically affect texture of broiler breast fillets (Dawson et al., 1987; Cavitt et al., 2004; Mehaffey et al., 2006; Brewer et al., 2012b, c). In this study, fillets deboned at 2 h PM had greater ($P<0.05$) shear values than fillets deboned at 6 h PM (Table 2). Moreover, fillets from the heritage birds had lower ($P<0.05$) shear values than either the high- or standard-yielding strains, regardless of deboning time however, shear values did not differ ($P<0.05$) between the commercial strains. Brewer et al. (2012b) reported significantly higher shear values for high-yielding strains grown to 60 d of age at 4 h PM, but, no difference at 6 h PM, which was similar to the findings in this study. It should be noted that for the standard and high yielding strains, despite the differences

between the 2 and 6 h debone, the average MORS energy for fillets from standard- and high-yielding birds would equate to sensory perception of "extremely tough" or "neither tough or tender" at 2 and 6 h PM (Cavitt et al., 2005). Regardless of debone hour and strain, both Mehaffey et al. (2006) and Brewer et al. (2012b) demonstrated that boneless breast fillets had MORS energy values equivalent to consumer evaluations of "tough" when birds were slaughtered at greater than 49 d of age. Conversely, MORS energy values for fillets from the random bred broilers would equate to consumers' perception of "very tender" (2 h debone) to "extremely tender" (6 h debone).

It should also be noted that this study was conducted to evaluate these strains at a fixed age not necessarily the most appropriate market age. Typically, random bred strain birds would be grown for an additional 4 weeks longer (to 12 weeks of age) than a commercial strain to reach an appropriate market size (approximately 4.5 lb.), and this could have some effect on tenderness. However, Fanatico et al. (2006) concluded that, even when raised to an appropriate market weight, consumers considered fillets from slow-growing more tender than fillet from commercial strains.

Fillets were cooked prior to shear analysis, and cook loss was evaluated in this study (Table 2) because of the close association with water-holding capacity and texture (Jeffery, 1983). Fillets from random bred broilers had less ($P<0.05$) cooking losses than fillets from high-yielding birds at 2 h PM and fillets form standard-yielding birds at 6 h PM. Mehaffey et al. (2006) reported similar findings for cook loss and shear values. Furthermore, cook loss percentage decreased ($P<0.05$) as deboning time increased from 2 to 6 h PM, regardless of broiler strain. Research has reported similar decreases in cook loss by extending debone hours

(Mehaffey et al., 2006; Brewer et al., 2012), and it can be attributed to increased interstitial space in the muscle for holding water when fillets are deboned later PM (Hedrick, 1994).

Muscle pH was measured, in this study, to monitor the progression of rigor, because the muscle becomes more acidic as rigor progresses with the accumulation of lactic acid (Hedrick, 1994). Even though *Pectoralis* pH values were within a normal range, pH of fillets from commercial strains decreased ($P<0.05$) from 2 to 6 h PM; however, fillet pH did not ($P<0.05$) change between 2 and 6 h PM (Table 3). Additionally, *Pectoralis* pH of both commercial strains was greater ($P<0.05$) than that of the heritage strain at both debone times, and fillet pH of the commercial strains was still greater ($P<0.05$) at 6 h PM than the pH of the random bred fillets at 2 h PM. This would indicate that birds with larger fillets (commercial strains) were progressing through rigor slower than birds with smaller breast fillets (random bred strain), similar to what was reported by Berri et al. (2001). Also, this could be the reason there was not a significant decrease in fillet pH for the random bred strain between 2 and 6 h PM, because rigor was nearing completion by 2 h PM and there was not a significant accumulation of lactic acid beyond 2 h PM. Between the commercial strains, there was not a significant difference at either 2 or 6 h PM. Indicating that between these two strains, there is not enough difference in fillet size or composition to affect PM muscle metabolism when measured at these times PM. It should also be considered fillets from commercial strains are considerably thicker ($P<0.05$) than fillets from random bred broilers. Taking this into consideration, differences in fillet thickness would potentially slow dissipation thereby extending rigor mortis development in commercial strains.

Toughness of broiler breast meat has traditionally been attributed to sarcomere shortening (Nakamura et al, 1975), and shortened sarcomeres have been reportedly shown to occur with deboning fillets prior to the completion of rigor (Lyon et al., 1985; Dawson et al., 1987;

Thompson et al., 1987). Although sarcomere lengths were similar ($P<0.05$) between commercial strains at both 2 and 6 h PM, sarcomeres were shorter ($P<0.05$) at 2 h compared to 6 h PM in the commercial strains (Table 3). Interestingly, fillets from heritage birds had longer ($P<0.05$) sarcomeres than the commercial strains when deboned at 2 h PM, but had shorter ($P<0.05$) sarcomeres than fillets from standard-yielding birds when deboned at 6 h PM. However, at 6 h PM the random bred strain had significantly shorter sarcomere lengths than the high-yielding strain0.05*(*$P<0.05$*)* and similar sarcomere lengths compared to the standard strain. Although sarcomere lengths for the random bred strain were the shortest numerically at 6 h PM, these fillets had the lowest ($P<0.05$) MORS force and energy values recorded of all fillets in this study. Size of fillets increase through hypertrophy and elongation of the muscle fibers (Hedrick, 1994); therefore, it is plausible that fillets form faster-growing, larger strains of broilers may be naturally longer than muscle fibers from smaller, slow-growing broiler strains. However, this degree of contraction was not addressed or evident in this study and would need to be confirmed using an earlier debone time for the random bred strain birds because, as *Pectoralis* pH indicates they are nearing rigor completion by 2 h PM. In this study, however, these data seem to indicate that although sarcomere length does play a significant role in fillet texture, it may not be the only contributing factor particularly between commercial and random bred strains.

Postmortem proteolysis plays a role in meat tenderness, particularly in red meats (Shackelford et al., 1994), and McKee et al. (1997) reported improvements in broiler breast fillet tenderness could be attributed to enzymatic weakening of the myofibrillar lattice. One was to estimate the extent of postmortem proteolysis is to measure the gravimetric fragmentation index (GFI), which would indicate the amount of muscle fragmentation occurring in breast fillets (Sams et al., 1991). Regardless of broiler strain, muscle fragmentation increased ($P<0.05$) from

2 to 6 h PM, as indicated by greater GFI values at 2 than 6 h PM (Table 3). Among strains, however, GFI values did not ($P<0.05$) differ at either deboning time. The calpain proteolytic system is primarily responsible for the normal turnover of skeletal muscle proteins, and μ-calpain is largely credited for PM tenderization (Koohmaraie, 1994). There is evidence that increased calpastatin activity- the endogenous inhibitor of μ-calpain- could result in increased shear force values (Shackelford et al., 1994). It could also be concluded that increased calpastatin activity would be required for animals that accrue greater muscle mass; therefore, perhaps, in these high-yielding broiler strains, the increased calpastatin activity, which potentially allows for greater breast fillet yield, also inhibits PM proteolysis, and could result in increased shear force for high yielding strains. However this was not evaluated in the current study and further research should be conducted to determine the effect of the calpain proteolytic system on broiler breast fillet tenderness.

There has been some indication that myofibrillar diameter could potentially affect meat texture (Smith and Fletcher, 1998; Crouse et al., 1990; Honikel and Reagan, 1986; Papa and Fletcher, 1988). It is also accepted that increased muscle yield and growth is commonly attributed to hypertrophy and elongation of the muscle fiber (Smith, 1963; Hedrick, 1994). Therefore, it could be assumed that high yielding strains could have greater muscle fiber diameter which could potentially increase shear force. Previous research has not necessarily confirmed this and it has also been hypothesized that increased breast fillet yield between strains could be attributed to myofibrillar number (Scheuermann et al., 2003). In this study, there was not a significant difference in myofibrillar diameter between the commercial strains. However, the random bred strain MD was significantly smaller than the commercial strains. Therefore, these data indicate that MD could be a contributing factor to fillet toughness between the

commercial and random bred strains; however, between the two commercial strains it is probably not a contributing factor because there was no difference in MORS energy of force and no difference in MD between commercial strains. Therefore the effect of MD on texture of birds in this weight range is not conclusive.

Conclusion

Meat texture, although it is a critical factor for consumer acceptance of poultry products, is difficult to define because of the many factors that can contribute to tenderness. In the current study, between debone hours, a change in sarcomere length is probably most likely the cause of differences in shear force. It should be considered that fillets from the commercial strains in this study are undergoing a greater degree of contraction compared to the lesser yielding random bred strain, and further research is needed in this area to determine the effect of this degree of sarcomere contraction. In this study, however, between commercial strains and random bred strains, it appears that sarcomere length is not the only cause of increased shear values. The results of this study suggest factors such as myofibrillar diameter may also play a role in meat tenderness of modern broilers. Therefore, the selection for greater breast yield (muscle fiber hypertrophy) has resulted in increased shear values of the breast fillet. Also, the role of the calpain proteolytic system in muscle development of standard and high yielding commercial broiler should be evaluated. The effect of this PM fragmentation of the breast muscle should also be addressed further, in particular the role of calpastatin as this could potentially be of value for improving tenderness of broiler breast fillets of the modern commercial broiler.

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Table 1. Stain main effect on live and ready to cook (RTC) weight and yield

	Live Weight (g)	RTC Weight (g)	RTC Yield %
Standard	3215[a]	2418[a]	74.8[b]
High-yielding	3050[b]	2307[b]	75.9[a]
Random bred	1360[c]	921[c]	67.7[c]
Pooled SEM	69.08	56.63	0.32

[a-c] Means lacking common superscript within attribute and 2 h debone are significantly different **(***P*<0.05**)**

[x-y] Means lacking common superscript within attribute and 6 h debone row are significantly different (*P*<0.05)

[*] Means between debone hour within strain are significantly greater (*P*<0.05)

[1] Fillet yield calculated as a percent of RTC weight

[2] Measured on cooked fillets during texture analysis

n=30/mean

Table 2. Interaction of strain and debone hour on fillet weight and yield

	Fillet Weight (g)		Fillet Yield %[1]		Fillet Thickness (mm)[2]	
	2 hour	**6 hour**	**2 hour**	**6 hour**	**2 hour**	**6 hour**
Standard	292.4^{a*}	259.8^{y}	11.9^{b*}	10.9^{y}	36.8^{a*}	33.2^{y}
High-yielding	284.8^{a}	281.4^{x}	12.4^{a*}	12.0^{x}	36.4^{a*}	35.3^{x}
Random bred	57.1^{b*}	51.0^{z}	6.1^{c*}	5.7^{z}	12.9^{b}	12.1^{z}
Pooled SEM	12.71		0.34		0.01	

$^{a-c}$ Means lacking common superscript within attribute and 2 h debone are significantly different **(***P*<0.05**)**

$^{x-y}$ Means lacking common superscript within attribute and 6 h debone row are significantly different (*P*<0.05)

* Means between debone hour within strain are significantly greater (*P*<0.05)

[1] Fillet yield calculated as a percent of RTC weight

[2] Measured on cooked fillets during texture analysis

n=30/mean

Table 3. Means for shear force, shear energy and cook loss of *Pectoralis* from 3 strains deboned at 2 and 6 h postmortem

	Shear Force (N)		Shear Energy (N.mm)		Cook Loss (%)	
Strain	**2 hour**	**6 hour**	**2 hour**	**6 hour**	**2 hour**	**6 hour**
Standard	15.9^{a*}	12.2^{x}	228.4^{a*}	168.0^{x}	28.5^{ab*}	27.3^{x}
High Yielding	17.0^{a*}	12.2^{x}	236.5^{a*}	162.2^{x}	29.2^{a*}	25.1^{xy}
Random bred	11.2^{b*}	8.1^{y}	121.6^{b*}	88.3^{y}	26.3^{b*}	24.5^{y}
Pooled SEM	3.16		12.45		0.19	

$^{a-c}$ Means lacking common superscript within attribute and 2 h debone are significantly different($P<0.05$)

$^{x-y}$ Means lacking common superscript within attribute and 6 h debone are significantly different($P<0.05$)

*Means between debone hour within strain are significantly greater ($P<0.05$)

n=30/mean

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December 2, 2013

To whom it may concern:

This certifies that Valerie Brewer serves as the first author on the paper, "Physical and biochemical properties effecting breast fillet tenderness of commercial and random bred strain broilers reared to 55 d." She completed over 51% of the associated research/work for the paper.

Casey M. Owens, Ph.D.
Major Professor and Co-author

TO: Casey Owens-Hanning
FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee
DATE: August 23, 2011
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: August 21, 2014

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12004- "PHYSICAL AND BIOCHEMICAL PROPERTIES ASSOCIATED WITH TENDERNESS IN BIG AND SMALL BIRD MARKET PROGRAMS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-14-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

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Chapter 6

Physical and biochemical properties associated with broiler breast fillet texture at two market ages

V.B. Brewer and C.M. Owens

Abstract

Commercial broilers are commonly reared to target the demands of 2 separate markets. To meet these demands, birds can either be reared to approximately 40 or 60 d of age. Previous research has indicated that that birds reared to 60 d of age will have greater shear force than birds reared to 40 d of age; however, the exact cause of this textural difference is not known. A study was conducted using commercial broilers reared to 40 and 60 d of age and deboned at either 2 or 4 h postmortem (PM) (n=25/age/debone hour). To evaluate the cause of textural differences gravimetric fragmentation, *Pectoralis* pH, myofibrillar diameter, and sarcomere length were evaluated. As was expected, birds reared to 60 d had greater ($P<0.05$) live weight, RTC yield, and fillet yield than birds reared to 40 d of age. There was a difference in shear for between debone hour for both ages, and 60-d-old birds deboned at 2 h PM were tougher ($P<0.05$) than any other treatment combination; however, tenderness was similar ($P<0.05$) between 40-d-old birds deboned at 2 h PM and 60-d-old birds deboned at 4 h PM. With the progression of rigor mortis there was no difference in pH at 2 h PM, but at 4 h PM pH, for 40-d-old birds had lower ($P<0.05$) pH values. At both debone hours, 60-d-old had greater ($P<0.05$) myofibrillar diameter than 40-d-old birds, and fiber diameter was greater at 2 than 4 h PM. For both ages, sarcomere length increased between 2 and 4 h PM, and interestingly, sarcomere lengths for 60-d-old birds were greater than 40-d-old bird at both debone hours. Fragmentation increased ($P<0.05$) between 2 and 4 h PM for both ages, but there was no ($P<0.05$) difference within debone hour for age. These results indicate several factors could be contributing to increased toughness between 40- and 60-d old broilers, including myofibrillar diameter and decreased rate of rigor

development; however, it does not appear that increased shear force can be attributed to sarcomere shortening.

Introduction

For the commercial broiler industry, multiple markets have emerged over the past few decades to better meet consumer demand and product specifications. To most effectively meet these market demands, birds are reared to different ages. Broilers reared to around 40 d of age are processed and used in the fast food market; so, breast fillet products can include both bone-in and boneless pieces. Birds reared to older ages (typically around 56 to 60 d of age) will typically be deboned and used in a variety of further processed products. It is not uncommon for birds for either of these market ages to be deboned as early as 2 h PM, because of the economic benefit associated with improved processing efficiency and yield. However, deboning carcasses at 2 h PM occurs prior to the completion of rigor, and can result in tough breast fillets (Lyon et al., 1973; Lyon et al., 1985; Dawson et al., 1987; Cavitt et al., 2005). It is commonly accepted that the toughness associated with early PM debone is caused by contraction and shortening of the sarcomere when there is still energy in the muscle (Stewart et al., 1984; Cavitt et al., 2004).

Previous research has indicated that bird age and size can affect meat quality; however, the exact cause is unknown (Cooper and Fletcher, 1997; Poole et al., 1999; Northcutt et al., 2001). Several studies have suggested that larger birds progress through rigor at a slower pace (Berri et al., 2001; Mehaffey et al., 2006; Fanatico et al., 2006; Brewer et al., 2012). This could mean that later postmortem (PM), there is still sufficient energy in the muscle to cause contraction, even up to 4 h PM. Also, it is well accepted that the muscle grows through hypertrophy of the muscle fiber as an animal increases in chronological age, and increased fiber

diameter has been linked to increased shear force in red meats (Acar et al. 1993, Schnaurmann et al., 2003; Tuma et al., 1962; Crouse et al., 1991; Shakleford et al., 1994). Finally, degree of postmortem proteolysis can affect meat texture. Research has indicated that in broiler breast fillets, PM proteolysis does improve meat tenderness; however, the difference in amount of myofibrillar fragmentation resulting from proteolysis between bird ages has not been evaluated. Therefore, the current study was conducted to measure the effect of rate of rigor, myofibrillar diameter, and PM fragmentation on boneless breast fillet texture of 2 different market ages.

Materials and Methods

For this study, commercial broilers were reared to 40 and 60 d of age (n = 50/age) for targeting these 2 common market ages. Feed, that met all NRC (1997) requirements, and water were provided *ad libitum* throughout grow-out. Feed was withdrawn approximately 10 h prior to processing. On the morning of processing broilers were cooped and transported to the University of Arkansas Pilot Processing Plant. The birds were slaughtered in 6 replicates (3 replicates/debone hour), with equal number of each bird age in each replicate, and commercially processed on an in-line system (Mehaffey et al., 2006). Immersion chilling was completed in 2 phases: Phase 1 consisted 0.25 h in 13^{o}C ice-water, and phase 2 consisted of 0.75 h in 4^{o}C ice-water. Following chill, carcasses were either immediately deboned (2 h) or they were well aged on ice until 4 h postmortem (PM).

Immediately post-debone, samples for *Pectoralis* pH were cut from the caudal tip of the right breast fillet, frozen in liquid nitrogen, and stored at -80C until further analysis. Single fillet weights were then taken with the left breast fillet for yield determination. Fillets were then packaged in zip-sealed bags, packed on ice, and stored in a cooler (4C) overnight until 24 h PM.

At 24 h PM, fillets were removed from cooler and samples were collected from the right breast fillet for sarcomere length determination, gravimetric fragmentation index, and myofibrillar diameter (MD). Samples for myofibrillar diameter were fixed in formalin, and other samples (-80C) and the left breast fillets (-20C) were frozen for further analysis.

Cook loss and shear values were determined on the left breast fillets. Briefly, fillets were removed from the freezer 24 h prior to cooking. Fillets were weighed and cooked on raised wire racks in aluminum foil covered pans at in a pre-heated (176 C) convection oven to an internal temperature of 76^{o}C. Shear energy (MORSE) and force (MORSF) were determined using the Meullenet Owens Razor Shear method (MORS), with a TA-XT2i texture analyzer (Texture Technologies, Scarsdale, NY), and 4 replicate shears were taken on each fillet.

For lab analysis, *Pectoralis* pH was determined using the iodoacetate method of Sams and Janky (1986), whereas gravimetric fragmentation index was determined using the method described by Sams et al. (1991). For myofibrillar diameter, samples were cut and fixed in formalin and subsequently stained with hematoxylin and eosin. Fiber diameter was measured using an Optronix microscope (20 x magnifications) and image analysis software, with 10 diameters measured on each sample. Finally, sarcomere length determination was conducted using the laser diffraction method of Voyle (1971), as modified by Cross et al., (1980), and 8 replicate sarcomeres were measured from each sample.

This experiment was analyzed as a complete random block design using the GLM procedure of SAS (SAS, 2002). Main effects in this study include bird age and debone hour. Processing replicate was analyzed for interaction, but was excluded from further analysis because it did not interact with either main effect. Differences were determined using Duncan's

Multiple Range Test and differences among treatment means were considered significant at $P<0.05$.

Results and Discussion

Broiler live weight, ready to cook (RTC) yield, and fillet yield were obviously greater ($P<0.05$) in 60-d-old than 40-d-old broilers (Table 1). Furthermore, live weights, fillet weights and yields were not affected by debone hour. Research has indicated that deboning breast fillets early can result in improved meat yield (Hirschler and Sams, 1998); however, this effect was not observed in the current study. .

Texture is the most important attribute for consumer acceptance of meat cuts (Morgan et al., 1991); therefore, the cause of textural differences between ages of broilers should be examined intensely. Textural differences due to debone hour and slaughter age were confirmed in the current study (Table 2). Debone hour affected fillet texture ($P<0.05$), with fillets deboned at 4 h PM having lower ($P<0.05$) MORS force and energy than fillets deboned at 2 h PM for both ages. This difference in MORS shear values between debone hours was expected due to the progression of rigor and decreased contraction of the muscle upon deboning when fillets are aged on the carcass for longer periods prior to debone. It is commonly accepted that rigor mortis will be complete in broilers around 4 h PM; thus by deboning after 4 h PM, fillet texture will not be affected by sarcomere contraction (Lyon et al., 1973; Stewart et al., 1984; Cavitt et al., 2004). Bird age also affected fillet tenderness in this study, whereas birds reared to 60 d had greater ($P<0.05$) MORS shear values than birds reared to 40 d regardless of debone hour. Furthermore, MORS values of fillets for the 40-d-birds deboned at 2 h PM were similar ($P>0.05$) to MORS shear values for fillets from 60-d-old birds deboned at 4 h PM. Northcutt et al. (2001) reported that fillet texture early PM was significantly affected by bird age; however, by 4 and 6 h PM,

there was no effect on fillet texture attributed to slaughter age. Based on these findings, the authors concluded that it was not necessary to age broiler carcasses past 4 h PM to prevent toughening of boneless breast fillets because there was no difference in fillet texture at any slaughter age between 4 and 6 h PM. The findings of Northcutt et al. (2001) are in contrast to the findings of the current study, which indicate that fillets from larger birds should be aged on the carcass for longer time PM to prevent increased toughness. Differences between studies could be largely attributed to different strains of commercial broiler used in these studies, and it should also be noted that several generations of selection for breast yield have occurred between Northcutt et al. (2001) and the current study. Results from the current study also indicate that younger, smaller birds can be deboned earlier PM than older, larger birds without the same implications on tenderness.

Pectoralis pH was similar ($P>0.05$) between 40- and 60-d-old birds at 2 h PM, but fillet pH was lower ($P<0.05$) in 40-d-old than 60-d-old broilers at 4 h PM (Table 3). These results suggest the *Pectoralis* of 60-d-old birds progresses through rigor at a slower pace than those of 40-d-old birds. Research has proposed that larger broiler breast muscles progress through rigor at a slower pace than smaller breast muscles due to lower glycolytic potential of the larger muscles (Berri et al., 2001; Bihan-Duval et al., 2001). If this is the case, then there could be sufficient energy in the muscle later PM to cause muscle contraction upon deboning resulting in tougher meat. Thus, results of the present study imply there is potentially energy left in the muscle at 4 h PM to cause sarcomere shortening and, in turn, increased shear values.

There is positive correlation between MORS energy and fillet height, with thicker fillets having greater MORS energy (Brewer et al., 2012). Also, research has indicated that fillets deboned early PM will be thicker than fillets deboned at later times PM (Brewer et al., 2009) due

to muscle shortening. In the present study, deboning time did not (P>0.05) affect fillet thickness for either bird age at 2 or 4 h PM. Again, sarcomere lengths were longer (P>0.05) at 4 h PM than at 2 h PM for both bird ages as would be expected with the progression of rigor. Interestingly, at both debone times, sarcomeres were longer (P>0.05) for the 60-d-old birds compared to the 40-d-old birds. Therefore, textural differences between ages in this study cannot solely be attributed to sarcomere length because the shortest sarcomeres (observed in 40-d-old birds deboned at 2 h) did not result in the toughest breast fillets (observed in 60-d-old birds at 2 h PM).

It would appear from these sarcomere length data that the contractile unit of the muscle for larger, older birds is longer than for younger, smaller birds. Research has suggested that increased fiber length could cause increased fillet yield between birds that are the same age (Acar et al., 1993, Schnauerman et al., 2003). Results from the current study suggest that, as bird age increases from 40 to 60 d, the muscle fiber is not only growing longer by increasing the number of sarcomeres/fiber, but perhaps the sarcomere itself is also increasing in length; moreover, it is plausible that, although the sarcomere lengths are longer for older birds, these sarcomeres are actually experiencing greater contraction compared to the smaller birds. It should be noted that there was greater percentage change in sarcomere length for the older compared to the younger birds (7.28% vs. 3.57 % change, respectively), supporting the hypothesis of greater degree of contraction upon early debone.

Myofibrillar diameter was measured because research has suggested that muscle will grows primary through hypertrophy and there is some indication in other species meat becomes tougher with increased fiber diameter (Tuma et al., 1962; Smith, 1963;Acar et al., 1993, Crouse et al., 1990). Within age, fiber diameter decreased (P>0.05) between debone hours for both bird

ages, and this would be expected because of increased overlap of the myofillaments for breast fillets deboned at 2 h PM. More importantly, fiber diameter of 60-d-old birds was 52.6 to 73.9% larger ($P>0.05$) than that of 40-d-old birds deboned at 4 and 2 h respectively. Size of fiber diameters observed in this study would indicate that much of the toughness associated with larger broilers may be attributed to the increased fiber diameter of the larger birds. Also, it should be considered that this degree of growth of the muscle fiber could have significant implications on the structure of the muscle potentially fillet texture, warranting further research.

Postmortem proteolysis can also affect meat tenderness, and often decreased yields associated with late PM debone have been attributed to PM proteolysis (Hirschler and Sams, 1998; Koohmaraie, 1994; Shakelford et al., 1993; McKee et al., 1997). Gravimetric fragmentation index (GFI) was measured in this study to evaluate PM fragmentation of the muscle, which is an indirect indicator of PM proteolysis. Within slaughter age, fragmentation increased ($P>0.05$) as the fillet was aged on the bone prior to deboning (Table 3), which concurs with the results of McKee and Sams (1997). Within debone hour, slaughter age did not, however, affect GFI.

Conclusion

Sarcomere shortening, as indicated by sarcomere length does not appear to be the cause of increased MORS shear values for the 60-d-old birds in this study. The degree of sarcomere contraction should be evaluated between bird ages because it is possible that there could be an effect of age on resting sarcomere length, so that older birds have longer sarcomeres. Although sarcomeres from older birds were longer at each debone time compared to younger birds, sarcomere shortening does not appear to be the underlying cause of increased MORS values of fillets from 60-d-old birds. Results of this study suggest the increased shear force observed for

fillet from larger broilers may be attributed more to slower progression of rigor development and increased fiber diameter than sarcomere length and PM proteolysis. Further research should be conducted to determine the effect of increased fiber diameter on muscle structure, including total and soluble collagen content.

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Table 1. Means for live weight, Ready to cook yield and fillet yield of broilers reared to 40 and 60 d of age

	Live Weight (g)	RTC Yield %	Fillet Weight (g)		Fillet Yield (%)[3,4]	
			2 hour	4 hour	2 hour	4 hour
40 d	1609.4[b]	72.8[b]	125.3[b]	116.7[b]	10.5[b]	10.1[b]
60 d	3338.6[a]	79.0[a]	324.7[a]	329.6[a]	12.5[a]	12.3[a]
Pooled SEM	95.1	0.4	16.4		0.14	

[a-e] Means lacking common superscript within one parameter are significantly different
[1] RTC yield calculated as percent of live weight
[2] Single fillet weight and yield
[3] Fillet yield calculated as percent of RTC weight
n=25/mean

Table 2. Means for shear force and shear energy of *Pectoralis* from 2 ages deboned at 2 and 4 h postmortem

	MORS Force (N)		MORS Energy (N.mm)		Fillet Height (mm)	
	2 hour	**4 hour**	**2 hour**	**4 hour**	**2 hour**	**4 hour**
40 d	16.1^{b}	14.5^{c}	219.4^{b}	189.3^{c}	23.2^{b}	22.1^{b}
60 d	19.4^{a}	16.8^{b}	246.1^{a}	208.1^{bc}	36.9^{a}	36.5^{a}
Pooled SEM	0.32		4.06		0.78	

$^{a-c}$ Means lacking common superscript are significantly different
n=25/mean

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December 2, 2013

To whom it may concern:

This certifies that Valerie Brewer serves as the first author on the paper, "Physical and biochemical properties effecting commercial broiler breast fillet texture at two market ages." She completed over 51% of the associated research/work for the paper.

Casey M. Owens, Ph.D.
Major Professor and Co-author

TO: Casey Owens-Hanning
FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee
DATE: August 23, 2011
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: August 21, 2014

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12004- "PHYSICAL AND BIOCHEMICAL PROPERTIES ASSOCIATED WITH TENDERNESS IN BIG AND SMALL BIRD MARKET PROGRAMS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-14-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

cnc/car

cc: Animal Welfare Veterinarian

Chapter 7

Effect of deboning time on breast fillet dimensions and prediction of breast fillet texture based on dimension

V.B. Brewer and C.M. Owens

Abstract

There is a high demand for boneless broiler breast meat, which has led to shortened aging periods in efforts to streamline production. It is well documented that pre-rigor deboning leads to shorter sarcomeres and tougher meat; therefore, the purpose of this study was to assess the impact of deboning time on fillet dimensions, and attempt to predict broiler breast fillet texture from these dimensions. Breast (*Pectoralis major*) fillets from 360 commercial broilers were deboned at 2, 4, and 6 h postmortem (PM; n=120/debone hour). *Pectoralis* pH was measured at debone time of debone using an intramuscular probe to monitor rigor progression, and fillet dimensions (length, width, and depth at 3 locations) were assessed using calipers at 24 h PM, and Meullenet-Owens razor shear method was used to evaluate texture. Debone hour did not ($P<0.05$) effect fillet weight in the current study, and width and thickness were correlated with fillet weight; so that fillets that were wider and thicker at the cranial portion also weighed more. Fillets became wider and thinner ($P<0.05$) as they aged on the bone prior to deboning, with the greatest changes in dimension noted for thickness measured at the middle and caudal locations. *Pectoralis* pH declined ($P>0.05$) at all PM times evaluated, and fillet texture ($P>0.05$) declined between 2 and 4 h PM, but not ($P>0.05$) between 4 and 6 h PM. Correlations of fillet dimension and meat quality parameters indicate no strong relationship of fillet dimension and *Pectoralis* pH. Although there were few relationships among fillet dimensions and MORS energy, fillet width had the strongest correlation with MORS energy, as indicated by a R^2 value of 0.50. Therefore, these results indicate that fillet dimensions are not good predictors of fillet texture, as measured by MORS energy.

Introduction

In the commercial poultry industry there is a wide variety of market bird sizes ranging from 2 to 8-plus pounds to meet specific consumer needs. Because meeting consumer need and maintaining profitability is critical, many processors are trending toward shorter aging periods prior to breast meat deboning. Furthermore, broiler breast meat is the most valuable and most domestically demanded part of the bird; therefore, broilers are grown to larger sizes to produce a greater amount of breast meat (weight and yield) per carcass also increasing profitability.

Lubritz (1997) concluded that greater fillet weight of commercial broilers can be attributed to increased fillet length, width, and thickness; however, fillet thickness had 7 times greater impact on this increased fillet weight than length or width. Several factors can influence fillet dimension, including strain, gender, feeding regimen, dietary amino acid density (Brewer et al., 2012 a, b; Dozier and Moran, 2002). Previous research on fillet dimensions, however, has used fillets that were deboned immediately after evisceration, without chill, or at one common debone hour. Lyon et al. (1997) reported no effect of deboning at 1, 2, or 3 h PM on the length and width of boneless breast fillets; however, all fillets were deboned earlier than the suggested debone time of 4 to 6 h PM (post-rigor) (Lyon et al., 1985, 1988; Stewart et al., 1988).

The effect of deboning prior to the completion of rigor on sarcomere shortening and fillet tenderness is well documented (Lyon et al., 1985; Papa et al. 1989; Herring 1967; Mehaffey et al., 2006; Cavitt et al., 2005; Brewer et al., 2012 c, d). Because of this, it could be assumed that sarcomere shortening following pre-rigor debone could potentially affect raw fillet dimensions due to the shortening of the sarcomere during contraction (Hanson and Huxley, 1954). Therefore, the effect of debone hour on fillet dimension could be of value to processors, especially when portioning fillets. Furthermore, previous research successfully evaluated the

progression of rigor using non-destructive methods (Cavitt and Sams, 2003), and fillet dimension could be a method to predict rigor development and boneless breast fillet texture. If breast fillet texture could be predicted early PM non-invasively, fillets could be allocated to more appropriate products and further processing techniques, such as marination, to compensate for increase toughness. Therefore, the objective of this study was to determine the effect of variable aging periods, both pre- and post-rigor on fillet dimensions as well as evaluate the potential of predicting breast fillet textural differences based on fillet dimensions.

Materials and Methods

A standard-yielding, commercial broiler strain was processed over 3 replicate processing days with 2 replicate debone hours/processing day. Broilers were processed at the University of Arkansas Pilot Processing Plant on an inline system (Mehaffey et al., 2006; Brewer et al., 2012). Following evisceration carcasses were immersion chilled for 75 min (15 min pre-chill at 13^{o}C; 60 min chill at 4^{o}C). Carcasses were well-packed and aged on ice until the appropriate debone time at 2, 4, or 6 h PM. Prior to deboning carcasses were weighed, and deboning was performed by 4 trained, experienced personnel. Breast fillets (*Pectoralis major*) weighed, and after weighing, fillets were packaged in zip-sealed bags, packed on ice, and held overnight in a 4^{o}C cooler.

Fillet dimensions were measured on the left breast fillet at 24h PM. Seven measurements were made on each fillet, including length at longest point, width at widest point, cranial thickness at thickest portion of the fillet, caudal thickness measured 2.5 cm from the bottom of the fillet, and thickness at the midpoint of the cranial and caudal measurements. After measurements, fillets were stored at -28^{o}C until measurement of cook loss and shear force. Fillets were removed from the freezer prior to cooking to thaw, and fillets were cooked to an end

point temperature of 76^{o}C in a preheated (177^{o}C)convection oven on wire racks in aluminum foil lined and covered pans (Sams, 1991; Mehaffey et al., 2006; Brewer et al., 2012). Fillets were cooled to room temperature and were then individually wrapped and stored overnight in a 4^{o}C cooler for texture analysis the following day. Meullenet-Owens razor shear method (MORS) (Cavitt et al., 2004; Mehaffey et al., 2006) was used to determine shear energy with 4 replicate shears taken per fillet.

Data were analyzed as a completely randomized design using the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). Debone hour was the main effect in the model; however, because straight-run broilers were used in the current study, gender was used as a random variable in analysis to account for potential effect on parameters measured. Differences among treatment means were established using Duncan's multiple range test, and means were considered significant at $P<0.05$. Pearson product correlations were evaluated to determine the relationship between fillet weight and dimension parameters, between growth parameters and *Pectoralis* pH and MORSE, and between fillet dimension and *Pectoralis* pH and MORSE. Relationships between fillet dimension and MORSE were modeled using linear regression (PROC REG) of SAS.

Results and Discussion

Growth parameters of live weight and fillet yield were evaluated in the current study however, will be excluded from discussion because of lack of significance between debone hours. Average fillet weight was 2.6 kg, which was 16.2% of the live broiler weight. Fillet weight can potentially affect fillet dimension and data for this parameter is presented in Table 1. Fillet weight did not ($P<0.05$) differ among debone times. Due to a of lack of significance for debone hour, debone hour was combined for correlation of fillet weight and live weight and

dimension parameters. Live weight and fillet weight were perfectly correlated (r=1.00); so, fillet weight increased similarly with increasing bird weigh. Fillet length, width, and cranial thickness were positively correlated with fillet weight (r > 0.70), indicating that fillets that weighed more are also longer, wider, and thicker at the cranial portion of the fillet, which is to be expected.

The effect of debone hour on fillet dimension could be of value to processors, particularly if fillets are being portioned for further processed products. Debone hour did have a significant effect on fillet dimension in this study with the exception of fillet length. Fillet width and cranial thickness were greater ($P<0.05$) when deboned at 2 than 4 h PM, but; fillet width and cranial thickness did not ($P<0.05$) change between 4 and 6 h PM. Middle and caudal thickness were thinner ($P<0.05$) at 6 h PM compared to 2 h PM, but were similar ($P<0.05$) between 2 and 4 h, and 4 and 6 h PM. These changes in fillet dimension were likely due to sarcomere shortening associated with early PM deboning of breast fillets (Papa and Fletcher, 1988; Cavitt et al., 2004). There was a greater percent change from 2 to 4 h PM for all parameters, except middle and caudal thickness where there was a greater change from 4 to 6 h PM. Also, considering total percent change from 2 to 6 h PM, middle and caudal thicknesses increased had the greatest total percent change of 14.4 and 18.1% respectively. These changes would potentially affect portioning processes and resultant products.

Fillet texture was analyzed in this study because texture is critical for consumer acceptance of meat products (Morgan et al., 1991), and perhaps because fillet dimension ($P<0.05$) changes significantly as fillets are aged on the bone it could be used to project broiler breast fillet texture. Also, *Pectoralis* pH was evaluated in this study because it can indicate PM progression of rigor. Debone hour had a significant effect on pH and shear values in this study (Table 1). *Pectoralis* pH declined ($P<0.05$) at each PM debone hour evaluated, as expected,

indicating that the rigor process is progressing normally between 2 and 6 h PM. As rigor progresses, there is less energy available in the muscle to cause sarcomere contraction. This sarcomere contraction is likely the cause of changes in fillet dimension, and has been traditionally accepted as the cause of toughness associated with broiler breast fillets deboned prior to the completion of rigor (Nakamura et al., 1975; Cavitt et al., 2004). In the current study, although pH declines significantly at all PM debone hours, fillet texture, as indicated by MORS energy only significantly changes between 2 and 4 h PM, but fillet texture did not (P>0.05) change substantially between 4 and 6 h PM.

To examine the relationship between fillet dimension and *Pectoralis* pH and MORS energy, Pearson Product correlations were evaluated and correlation coefficients and P-values are presented in Table 3. Only fillet width produced a significant correlation (r = -0.49) with *Pectoralis* pH. Although it may appear that middle and caudal thickness would be a predictor of fillet texture because these parameters experience the greatest percent change as fillets age on the bone, these dimensions were lowly correlated (r = 0.07 and 0.33, respectively) with MORS energy. Fillet length (r = -0.57) and width (r = -0.70) produced significant correlations with MORS energies.

Regression modeling indicated that fillet weight and thickness were not strong predictors of MORS energy ($R^2 \leq 0.15$; Table 4) statistics were used to further evaluate any potential predictive value of fillet dimension for MORS energy (Table 4). However, fillet length accounted for 33% of the variation in MORS energy, whereas fillet width explained 50% of the variation in MORS energy; however, when combined, the predictive accuracy of fillet width and length decreased ($R^2 = 0.29$) To evaluate this effect length, width, and cranial thickness were evaluated; also, in an to attempt to correct for differences in individual variability in fillet size,

dimension parameters were divided by fillet weight, length and width where appropriate. These analyses indicate that none of the parameters evaluated had strong predictive value for MORS energy due to low R^2 values. The dimension parameter with the greatest R^2 value evaluated was fillet width ($R^2 = 0.50$), when left uncorrected for fillet size. Because of this, it can be concluded that fillet dimension cannot be used to predict textural properties of boneless breast fillets across debone hour. Furthermore, it should be noted that attempts to predict tenderness within debone hour based on fillet dimension were also unsuccessful.

Conclusion

In conclusion, breast fillet dimensions change when breast fillets are deboned later PM compared to early PM. However fillet weight and thickness were not good predictors of breast fillet texture. Conversely, fillet width singularly accounted for 50% of the variation in MORS energy, and shows promise as an early PM predictor of cooked fillet tenderness. In general, the lack of predictability of texture based on fillet dimension may be related to the natural biological variation in muscle size from bird to bird. Many factors also affect breast dimension s including genetics, growth performance (nutrition and management), age etc.; therefore, the amount of variability from flock to flock would also be difficult to overcome in using breast fillet dimension as a predictive variable.

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Table 1: Boneless breast fillet weight[1], dimension and meat quality parameters from broilers grown for further processed market at 3postmortem debone times

					Percent Change[6]		
	2	4	6	Pooled	2 to 4	4 to 6	2 to 6
Attribute	**hour**	**Hour**	**Hour**	**SEM**	**hour**	**hour**	**hour**
Fillet Weight (g)	249.7a	248.7a	248.2[a]	6.44			
Fillet Length[2]	18.6[a]	18.9[a]	19.4[a]	0.07	1.61	3.17	4.84
Fillet Width[2]	9.14[b]	9.61[a]	9.79[a]	0.05	4.92	2.29	7.33
Cranial Thickness[2]	2.90[a]	2.74[b]	2.67[b]	0.02	6.21	1.84	7.93
Middle Thickness[2]	1.87[a]	1.75[ab]	1.60[b]	0.02	8.02	6.98	14.4
Caudal Thickness[2]	1.16[a]	1.07[ab]	0.95[b]	0.02	7.76	11.21	18.1
MORSE (N.mm)	191.3[a]	162.4[b]	159.6[b]	2.07	15.10	1.72	16.57
***Pectoralis* pH**	6.43[a]	5.92[b]	5.77[c]	0.02	7.93	2.53	10.26

[a-c] Within row means lacking common subscript are significantly different ($P<0.05$)

[1] Butterfly breast fillet

2 Measurements taken at 24 h PM on raw breast fillets

3 Cook loss calculated as a percentage of pre-cooked weight

4 Percent change calculated as a percent of initial measurement, therefore percent change from 2 to 4 hour is calculated as a percent of 2 h dimension, 4 to 6 h dimension calculated as percent of 4 h dimension

N=120 per debone hour

Table 2: Pearson correlation coefficient[1] of growth and dimension parameters for broiler grown for further processed market

	Fillet Weight[3]
Live weight (g)	1.00 (0.00)
Fillet Length[2]	0.58 (0.09)
Fillet Width[2]	0.80 (0.00)
Cranial Thickness[2]	0.82 (0.00)
Mid-point Thickness[2]	0.55 (0.01)
Caudal Thickness	0.42 (0.07)

[1] Correlations calculated on replicate mean data (n=20 per replication)
[2] Measurements taken at 24 h PM on raw breast fillets
[3] *P*-value in parentheses

Table 3: Pearson correlation coefficient[1] *Pectoralis* pH and MORS energy of broilers reared for further processed market

	***Pectoralis* pH[3]**	**MORS Energy[3]**
Live weight (g)	-0.14 (0.57)	-0.39 (0.10)
Fillet Weight (g)	-0.14 (0.57)	-0.39 (0.10)
Fillet Length[2]	-0.32 (0.20)	-0.57 (0.01)
Fillet Width[2]	-0.49 (0.04)	-0.70 (0.00)
Cranial Thickness[2]	-0.06 (0.83)	0.11 (0.65)
Mid-point Thickness[2]	0.13 (0.61)	0.07 (0.78)
Caudal Thickness[2]	0.45 (0.06)	0.33 (0.18)
MORS Energy	0.62 (0.01)	NA

[1] Correlations calculated on replicate mean data (n=20 per replication)
[2] Measurements taken at 24 h PM on raw breast fillets
[3] P-value in parentheses

Table 4: Regression model statistics for MORS energy from fillet attributes

	MORS Energy	
Fillet Attribute	**RMSE**	**R^2**
Weight	21.26	0.15
Length	18.98	0.33
Length/Weight	21.89	0.11
Length/Width	19.47	0.29
Width	16.38	0.50
Width/Weight	23.09	0.01
Width/Length	19.50	0.29
Thick	23.03	0.01
Thick/Weight	19.01	0.32
Thick/Length	22.91	0.02
Thick/Width	19.47	0.29

[1] Regressions calculated on replicate mean data (n=20 per replication)

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December 2, 2013

To whom it may concern:

This certifies that Valerie Brewer serves as the first author on the paper, "Effect of deboning time on breast fillet dimension and prediction Of breast fillet texture based on dimension." She completed over 51% of the associated research/work for the paper.

Casey M. Owens, Ph.D.
Major Professor and Co-author

TO: Casey Owens-Hanning
FROM: Craig N. Coon, Chairman
Institutional Animal Care and Use Committee
DATE: August 23, 2011
SUBJECT: IACUC PROTOCOL APPROVAL
Expiration date: August 21, 2014

The Institutional Animal Care and Use Committee (IACUC) has **APPROVED** Protocol **#12004- "PHYSICAL AND BIOCHEMICAL PROPERTIES ASSOCIATED WITH TENDERNESS IN BIG AND SMALL BIRD MARKET PROGRAMS** ". You may begin this study immediately.

The IACUC encourages you to make sure that you are also in compliance with other UAF committees such as Biosafety, Toxic Substances and/or Radiation Safety if your project has components that fall under their purview.

In granting its approval, the IACUC has approved only the protocol provided. Should there be any changes in the protocol during the research, please notify the IACUC in writing [Modification Request form] **prior** to initiating the changes. If the study period is expected to extend beyond **08-14-2014,** you must submit a new protocol. By policy the IACUC cannot approve a study for more than 3 years at a time.

The IACUC appreciates your cooperation in complying with University and Federal guidelines for research involving animal subjects.

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Chapter 8

Conclusions and Future Research

Pleasing texture of boneless breast meat is critical for consumer satisfaction and therefore is a concern for the commercial poultry industry. However, demands for efficiency and profitability have resulted in the common industry practice of deboning meat early PM (approximately 1.75 to 2 h PM) despite the fact that it is commonly accepted that this practice will result in toughening of breast fillets. However, these data presented here suggest that there are other factors contributing to increased shear values of older, larger birds compared to smaller, younger birds. Studies presented here that had age as a main effect, showed that regardless of debone hour fillets from older birds were tougher than fillets from younger birds. Also, it appears that would require approximately 3 additional hours of aging on the carcass (until 6 h PM) for fillets from large birds to reach equivalent tenderness of early PM deboned (3 h PM) small birds. Furthermore, even when deboning at 24 h PM boneless fillets from large broilers have similar shear values to fillets from small birds deboned at 3 h PM, and shear values of older broilers (52 d and 60 d of age) did not significantly decline beyond 3 h PM. Moreover, it has been accepted that marination can be used to improve boneless breast fillet texture. However, although breast fillet texture was improved with the use of tumble marination, shear values from marinated fillets only equated to consumer tenderness ratings of "slightly tough" for fillets deboned at 2 h PM.

The ability to predict boneless breast fillet tenderness in a raw state would result in the ability to allocate fillets that are tougher to products where more further processing steps could alleviate toughness. For example, if it could be predicted that a fillet was going to be unacceptably tough then it could be allocated to a product that used a higher percentage of salt in the marinade or a product that was portioned, injected, and tumble marinated. However, if it was predicted that a fillet was going to be less tough it could be allocated to a product that used a lesser salt percentage or underwent fewer processing steps. It was attempted to project fillet

texture based on fillet dimension, and proved unsuccessful although it does appear that fillet width would be the most promising dimension to evaluate to project fillet texture. Although dimension does not appear to be an appropriate method for evaluating fillet texture, other non-destructive predictive measures should be evaluated.

Several potential causes of this toughness were investigated in these studies. It does appear this toughness seems to be associated with some factor associated with selection for breast meat yield, because data presented here suggest commercial strains that have been selected for breast yield are significantly tougher than an unselected random bred strain, and although it does appear that larger broilers (older and commercial broiler strains) are progressing through rigor at a slower pace than small birds (random bred strain and younger commercial broilers), and this could potentially be a cause of the increased shear values early PM, this would not explain the increased shear values late PM (24 h PM) well beyond the completion of rigor mortis. To further confirm this sarcomere length data indicate that the shortest sarcomeres do not always result in the toughest meat. For example, the random bred strain birds had the shortest sarcomeres and the lowest shear values compared to the commercial strains, and 40 d old broilers deboned at 2 h PM had shorter sarcomeres and lower shear values ($P<0.05$) than 60 d old broilers deboned at 2 h PM. The effect of fillet size on sarcomere length warrants further investigation to determine if, in fact, sarcomere length is not a contributing factor to increased shear properties. A serial slaughter study should be conducted to determine if sarcomeres are actually longer in larger muscles from older birds, and therefore, are potentially undergoing an equivalent or greater degree of contraction compared to younger birds or birds with smaller muscles.

In evaluating myofibrillar fragmentation between bird ages and strains it does not appear that fragmentation plays a role within debone hour in textural differences. The effect of fragmentation however, should be evaluated for effects among other commercial strains. Also, the effect of the calpain proteolytic system on breast fillet yield and in turn fillet texture warrants investigation, because with large broilers there is not a significant change in fillet texture beyond the completion of rigor mortis perhaps indicating a greater calpastatin activity in larger broilers.

Data here indicate that the most likely cause of toughness associated with older larger birds is myofibrillar diameter. While this may not be the case between commercial strains that are the same age there is a significant difference (38% difference) in myofibrillar diameter between commercial strains and random bred strain broilers. Between slaughter ages evaluated here the myofibril is growing significantly (>50%), therefore, it should be considered not only the greater resistance to shear of the larger myofibrils but also the amount of muscle accretion and turnover that could be effecting tenderness and warrants further investigation; particularly the effect of this muscle growth on the structure and solubility state of the connective tissue surrounding the muscle fiber. It has to be assumed that the endomysium surrounding the muscle fiber is undergoing some change to compensate for the extreme growth over a short period of time. Therefore, it could be that a combination of increased myofibrillar diameter and decreased collagen solubility that are resulting in increased shear values.