Characterization and Consumer Acceptance of White Striping in Broiler Breast Fillets

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CHARACTERIZATION AND CONSUMER ACCEPTANCE OF WHITE STRIPING IN BROILER BREAST FILLETS
CHARACTERIZATION AND CONSUMER ACCEPTANCE OF WHITE STRIPING IN BROILER BREAST FILLETS

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Poultry Science

By

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ABSTRACT

White striping is an emerging issue in the broiler meat industry which causes concerns among the producers. The condition is characterized by the occurrence of white striations, parallel to the direction of muscle fibers, mainly in broiler breast fillets and thighs. Fillets can be categorized as NORM (normal or no striping), MOD (moderate) and SEV (severe) depending on the degree of white striping. As a result of few previous research studies on the condition, there is no information available on the impact of white striping on the consumer acceptance and various meat quality attributes. Our hypothesis was that the occurrence of the condition could be associated with tissue changes in meat and the physical appearance of the striations on the meat may affect the purchase intent of the product. The present study was intended to determine the consumer acceptance, meat quality and pathological changes associated with different degrees of white striping. The effects of growth rate, strain, gender, and dietary vitamin E level on the occurrence of condition were also evaluated. The results from the present study showed that MOD and SEV degrees of white striping reduced ($P < 0.05$) the consumer acceptance of the broiler breast fillets due to its marbled or fatty appearance. However, the condition did not show ($P > 0.05$) any effect on cooked meat quality attributes, mainly tenderness. Serologic profile and the histopathological findings indicated chronic myopathic tissue changes associated with higher degrees of white striping. These tissue changes were also manifested as increase in fat ($P < 0.05$) and decrease ($P < 0.05$) in protein contents as the degree of white striping increased from NORM to SEV. The occurrence of MOD and SEV degrees was closely associated ($P < 0.05$) with heavier birds or increased growth rate in broilers. Nonetheless, the incidence of the condition showed neither any strain/gender predilection nor relation to dietary vitamin E level. In conclusion, white striping is a growth associated myopathic condition seen in fast growing
broiler birds which can affect the consumer acceptance and proximate composition of broiler breast fillets.
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DEDICATION

To the two wonderful people – my wife, Anjana and daughter, Dhwani, who came to my life while in Arkansas making those days even more unforgettable.
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I. INTRODUCTION

Poultry meat has been highly preferred among the meat consumers mainly due to its health benefits, convenience in cooking and the reasonable cost (Haley, 2001; Davis and Stewart, 2002). The per capita consumption of poultry meat almost doubled during the last few decades (National Chicken Council, 2011a). As a result, the poultry industry underwent a rapid development. The success and sustainability of an industry depends on effectively reducing the gap between the producers and consumers in terms of supply and demand in the market. Therefore, increasing consumer demands has put pressure on the producers to increase production and reduce the cost and time of production. Birds are being continuously selected to attain a greater body weight at a younger age. In 1925, the average market live weight of a 112 d broiler was 2.5 lb while, in 2010, it increased to 5.8 lb attained in 47 d (National Chicken Council, 2011b). Furthermore, the consumer interest shifted from a whole bird carcass to cut up parts which favored the big bird processing sector. Now-a-days, broilers of > 4.5lb of live weight constitute around 77% of birds processed (National Chicken Council, 2005). The fast growing generation of modern birds can effectively meet the quantitative needs on the consumers. But, it is equally important to maintain the quality of the product as well.

When a product like boneless skinless broiler breast fillets is considered, appearance is the single and the key factor which decides the purchase intent. Recently, poultry meat producers have noticed the occurrence of white striations on broiler meat. The condition is referred to as white striping which is characterized by the occurrence of white lines, running parallel to the direction of muscle fibers, mainly seen on chicken fillets and thighs. The intensity of the condition may vary even in birds from the same flock. Since the white striping can be easily visible on raw breast fillets, the condition could have significant effect on the appearance of the
meat. Even though, the gross appearance of the meat with white striping seems similar to nutritional myopathy (Dam et al., 1952; Machlin and Shalkop, 1956) or muscular dystrophy (Julian and Asmundson, 1963; McMurtry et al., 1972), the pattern of the incidence seems to be different. However, the lack of any previous research on the specific condition makes it difficult to compare it with any known prior or existing conditions. Furthermore, the occurrence of white regions associated with condition could also remind the marbling in beef where it is considered as a superior quality trait (USDA-FSIS, 2008). So, it is necessary to characterized the white striping and determine its impact on poultry meat industry. The hypothesis of the present study is that white striping may have a significant effect on the consumer acceptance and also the proximate composition of poultry meat. Most important aim of the current research is to know the consumers’ opinion about white striping and how are they going to respond to poultry meat with white striping seen in grocery stores. It is also important to evaluate the tissue changes associated with the condition so as to determine its impact on the proximate composition and various meat quality parameters. Moreover, the characterization of white striping will assist in determining its similarity or dissimilarity with the known conditions seen in poultry. These findings may help us to identify the factors associated with white striping which in turn will provide suggestions regarding the etiology of the condition.
A. REFERENCES


II. REVIEW OF LITERATURE

Skeletal, smooth and cardiac muscles are the three types of muscle in an animal body. Among these, skeletal muscle constitutes a major portion of meat consumed as food (Forrest et al., 1975). Skeletal muscle mainly consists of muscle fibers and connective tissue. The muscle fibers, which are the elongated, multinucleated, non-branched skeletal muscle cells, are arranged in the framework of connective tissue matrix (Judge et al., 1989; Lawrie, 1998; Swartz et al., 2009). The connective tissue layers which envelope the muscle fibers, muscle bundles and the whole muscle are known as endomysium, perimysium and epimysium, respectively (Forrest et al., 1975). These three layers of connective tissue provide the platform for the nerve and vascular supply to the muscle (Swartz et al., 2009). The connective tissue also harbors adipocytes which are the cells assigned for storage of fat. The intramuscular fat or marbling is the fat deposited mainly in the perimysium while, intermuscular or seam fat is seen associated with epimysium (Judge et al., 1989). However, skeletal muscle may show a wide variation in gross anatomical, microscopic and subcellular properties depending on the species, strains, anatomical location, stage of development or even in samples obtained from different parts of the same muscle (Mahon et al., 1984; Mahon, 1999). Variation in the meat color can also be attributed from various other factors like geographical regions, seasons, diet, preslaughter and processing conditions (Smith and Northcutt, 2009). In addition, Valentine and McGavin, (2012) mentioned that the occurrence of pale streaks on muscle may be an indication of muscle damage leading to myofiber necrosis followed by mineralization or infiltration of collagen or fat. So, the primary need is to determine whether the occurrence of white striping is within the normal range of variations seen in skeletal muscle or is it a manifestation of abnormality due to some pathological condition affecting the normal arrangement of the muscle tissue. A detailed
knowledge of the structure, function and development of the muscle tissue as well as the normal physiological response to skeletal muscle damage is necessary to evaluate the tissue changes in white striping and to determine the underlying pathology, if any. Furthermore, a review of the existing muscular abnormalities in poultry and their impacts on the meat quality will assist to implement better approaches to identify the factors associated with the condition.

A. MUSCLE STRUCTURE AND FUNCTION

Muscle fibers are the basic structural unit of the living muscle tissue as well as meat (Swatland, 1994; Lawrie, 1998). Muscle fiber consists of plasma membrane (sarcolemma), cytoplasm (sarcoplasm), nucleus and other cellular organelles. In addition, muscle fibers also contain a unique organelle known as myofibrils which are long thin cylindrical rods seen in the sarcoplasm and extend almost the entire length of the muscle fiber (Forrest et al., 1975). The myofibrils are in turn composed of parallel arrangement of thin and thick myofilaments, known as actin and myosin, respectively (Allen and Goll, 2003). Actin and myosin filaments are arranged in an overlapping manner along the longitudinal axis of myofibrils (Swartz et al., 2009). This gives the characteristics alternating light and dark bands and hence the name striated muscle. The light band is known as I (isotropic) band and the dark band is known as A (anisotropic) band. A thin dark band that bisects the I band is referred to as the Z line (Forrest et al., 1975). The distance between the two adjacent Z lines of a myofibril is called the sarcomere which is the basic contractile unit where the contraction-relaxation cycle occurs in the muscle tissue (Forrest et al., 1975; Allen and Goll, 2003). Besides the contractile proteins, actin and myosin, the myofibril also contain various regulatory proteins like tropomyosin, troponin which are involved the regulation of muscle contraction (Forrest, 1975). The contractile proteins along
with the regulatory proteins constitute the myofibrillar proteins while the other types of proteins present in muscle fiber are sarcoplasmic and connective tissue proteins (Lawrie, 1975).

The nerve impulse for muscle contraction will result in a cascade of changes which will result in the formation of cross bridges between the actin and myosin filaments (Forrest, 1975). Later, at the expense of energy (ATP), the myosin filaments will pull the actin filaments towards the center of sarcomere resulting in contraction of muscle. Once the impulse is lost, the cross bridges formed between actin and myosin will be released resulting in relaxation of the muscle (Allen and Goll, 2003). During all phases of contraction, the width of the A band will be constant while that of I band changes which in turn affects the length of the sarcomere. So, the length of sarcomere mainly depends on the state of contraction and it has been found to have a great impact on meat tenderness (Forrest et al., 1975).

**B. GROWTH AND DEVELOPMENT OF MUSCLE TISSUE**

Growth is an important aspect of animal production. Producers always want to increase the growth rate of animals especially in meat production. According to Forrest et al. (1975), growth of a tissue can occur due to hypertrophy (enlargement of existing cells), hyperplasia (multiplication or production of new cells) or accretionary growth (increase in non-cellular structural material). So, there could be true growth (increase in structural tissues of muscle, bone and vital organs) or fattening (increase in adipose tissue). The growth of an animal can be divided into prenatal and post natal stages (Forrest et al., 1975; Rehfeldt et al., 2004). During the early prenatal periods, the growth is mainly due to hyperplasia of the muscle fibers although there will be hypertrophy of muscle fibers towards the later stages of prenatal development (Forrest et al., 1975; Rhoads et al., 2009). The prenatal growth starts from the fertilization of
ovum to form zygote which later develops into embryo and fetus. The zygote then undergoes a stage of rapid mitotic cleavage. Eventually, there will be formation of three layers of primary germ cells the ectoderm, endoderm and the mesoderm (Forrest et al., 1975). Muscle fibers originate from somite (a cluster of mesodermal cells found on each side of the neural tube of the developing embryo) as the myogenic precursor cells, which will undergo mitotic divisions to form myoblasts, align into chains and fuse together to form myotubes (Allen and Goll, 2003). Then, the myotubes synthesize various muscle proteins and ultimately differentiate to muscle fiber (Sinowatz, 2010). However, all the muscle cells do not develop at the same time; rather various subpopulations of the myogenic cells undergo different stages of development (Allen and Goll, 2003; Sinowatz, 2010). One branch of the myogenic cells undergoes development relatively earlier to form primary fibers. Another class of myogenic cells develop later to form secondary fibers. Later, a third branch of the myogenic cells gives rise to the satellite cells which are involved in post natal muscle development (Allen and Goll, 2003).

The postnatal growth of muscle tissue occurs mainly through hypertrophy (Forrest et al., 1975; Rhoads et al., 2009). In addition, there will be maintenance and repair of the damaged muscle tissue in the animal. Mauro (1961) first reported the presence of a quiescent muscle progenitor cell which is associated with post natal growth and regeneration of muscle tissue. These cells are seen “wedged” between the plasma membrane of the muscle fiber and the basement membrane, intimately associated with the muscle fiber and hence he used the termed satellite cells (Mauro, 1961). Postnatal myogenesis mediated through satellite cells is almost similar to that of the embryonic muscle development (Wang and Rudnicki, 2012). Satellite cells are mitotically dormant in normal muscle tissue. When stimulated by exercise, injury or muscle degeneration, the satellite cells become activated and undergo proliferation to form myoblast
(Hawke and Garry, 2001; Wang and Rudnicki, 2012). Later, a portion of these cells will undergo terminal differentiation and fuse with the existing muscle fiber or fuse together to form new myofibers while rest of the activated satellite cells will get recruited to maintain the satellite cell pool in the muscle tissue (Collins et al., 2005; Montarras et al., 2005; Kuang et al., 2007).

C. SKELETAL MUSCLE RESPONSE TO DAMAGE

Damage of muscle tissue can occur because of physical, toxic, ischaemic, nutritional deficiency, infective or immunological or genetic reasons (Mahon, 1999). Once damaged, the body has to repair the tissue in order to establish the homeostasis. Homeostasis refers to the ability and tendency of a system to maintain a physiologically balanced internal state under various environmental imbalances (Forrest et al., 1975). The maintenance of homeostasis is necessary for the proper functioning of various organ systems in the animal body. In order to reestablish the homeostasis, the damaged skeletal muscle undergoes different overlapping stages of reparative processes such as degeneration, inflammation, fibrosis and regeneration (Figure 1) irrespective of the source of damage (Prisk and Huard, 2003; Järvinen et al., 2005). During a trauma or damage, the integrity of the myofibrils are lost resulting in the entry of extracellular ions like sodium and calcium (Carpenter and Karpati, 1989) as well as the initiation of the complement cascade in the skeletal muscle (Orimo et al., 1991). Eventually, the excess influx of the extracellular calcium and/or the increased release calcium from the intracellular stores will activate intracellular proteases or lipases and/or disrupt the mitochondrial function leading to degeneration and necrosis of the muscle fibers (Jackson et al., 1984; Mahon, 1999; Alderton and Richard, 2000; Sandercock and Mitchell, 2003; Mitchell and Sandercock, 2004; Whitehead et al., 2006; Millay et al., 2009). The inflammatory phase involves the invasion of the damaged area by neutrophils, activated macrophages and T-lymphocytes (Prisk and Huard, 2003; Smith et
al., 2008). These cells are mainly associated with the phagocytosis of cellular debris and the release of factors like cytokines, prostaglandins etc., which are involved in either the degeneration or regeneration of muscle fibers and also the formation of fibrous tissue (Prisk and Huard, 2003; Smith et al., 2008). Also, the inception of muscle fibers regeneration is through the activation and proliferation of the satellite cells, located between the sarcolemma and basal lamina, due to the action of the factors released at the injury site (Kääriäinen et al., 2000). Later, these cells will proliferate and either fuse together and differentiate to form multinucleated mature myofibers or maintain the satellite cell pool (Zammit et al., 2004; Collins et al., 2005; Montarras et al., 2005; Kuang et al., 2007). Meanwhile, the development of fibrosis (type III collagen followed by type I collagen) at the site of muscle damage acts as platform for the formation of new myofibers (Kääriäinen et al., 2000; Prisk and Huard, 2003). The fibrous tissue scaffold also plays an important role in the initiation of blood supply and formation of the neuromuscular junctions in the newly formed muscle tissue. After the regeneration of the damaged tissue, the components of extracellular fibrous tissue will be degraded as a result of the controlled action of proteases (Mann et al., 2011). Thus, a normal homeostasis of the system will be reestablished. However, variations in regenerative properties can be seen because of genetic, hormonal, neural factors or due to differences in age and vascular supply (Mahon, 1999).

Pathological changes are only exhibited when the muscle damage is “too great”, “too acute” or “too continuous” for the muscle tissue to regenerate or repair (Mahon, 1999). In this case, the regenerative phase will be inefficient and there will be irreversible changes in homeostasis accompanied by accumulation of fat and fibrous tissue leading to fatty degeneration (Natarajan et al., 2010). However, there exist controversies about the sources of the adipocytes and fibroblasts seen in fatty degeneration. A number of studies reported that the satellite cells are
pluripotent to differentiate into myotubes, adipocytes, fibroblasts or osteoblasts (Asakura et al., 2001; Wada et al., 2002; Shefer et al., 2004; Brack et al., 2007) and the fate could be determined by its interaction with the degenerating muscle fiber (Hosoyama et al., 2009). On the other hand, some of the recent studies identified the presence of a novel type of cells in skeletal muscle which are involved in skeletal muscle response to damage (Joe et al., 2010; Uezumi et al., 2010; 2011). These cells are referred to as fibro/adipogenic progenitors (FAP) because of their capability to differentiate either to myofibroblasts or to adipocytes. As a result of the muscle damage, the FAP cells will get stimulated, even before the myogenic satellite cells, and enter into cell cycle (Natarajan et al., 2010). They also produce signals which cause the differentiation of satellite cells thus promoting the regeneration process (Joe et al., 2010). Later, the fate of the FAP cells depends upon the progression of muscle regeneration. Uezumi et al. (2010) showed that the direct contact with regenerating myofibers formed from the stimulated satellite cells will inhibit the differentiation of FAP cells into adipocytes. Therefore, at the end of an efficient regenerative process, the FAP cells will undergo apoptotic cell death. Meanwhile, the absence of regenerating myofibers, as in the case of an unsuccessful regenerative process, will lead to enhanced differentiation of FAP into adipocytes and fibrous tissue resulting in fatty degeneration (Natarajan et al., 2010). However, the actual signals that stimulate reparative function of the FAP cells and the existence of any similar progenitors in skeletal muscle are still uncertain (Natarajan et al., 2010). The above tissue changes associated with muscle damage will be reflected in plasma or serum profile as well. The damage of muscle tissue disrupts the integrity of sarcolemma which causes the leaking of a number of enzymes which are often used as the clinical indicators of the type, extent and origin of the lesions (Mitchell, 1999). Most commonly used muscle damage indicators are creatine kinase (CK), aspartate aminotransferase (AST) and
lactate dehydrogenase (LDH). However, it is always difficult to set a normal range for the serum/plasma level of these enzymes as it may vary between and within laboratories (Valentine and McGavin, 2012). Though, the tissue changes explained above are based on studies conducted on mammalian species, most of the biochemical changes taking place in avian muscle are comparable to mammalian muscle with subtle differences (Dutson and Carter, 1985).

D. CONVERSION OF MUSCLE TO MEAT

Muscle has to undergo a series of changes postmortem so as to get converted to meat. First event taking place during the processing of a meat animal is circulatory failure, mainly due to exsanguination. As a result, the transportation of nutrients to the muscle tissue and the removal of waste products from the muscle tissue are eliminated and the homeostasis is disturbed (Forrest et al., 1975; Lawrie, 1998). In order to mainly the homeostasis, the muscle tissue will try to maintain the structural integrity by continued production of energy (Lawrie, 1998). Since blood supply is lost, there will not be the supply of oxygen anymore. So, the muscle will shift from an aerobic to anaerobic metabolism to meet the demand for energy. Creatine phosphate and glycogen stored in the muscle are used as the source of energy to generate ATP (Lawrie, 1998). The lactic acid produced from anaerobic metabolism begins to accumulate in the muscle resulting in the lowering of muscle pH (Forrest et al., 1975). The process continues until nearly all the glycogen stored in the muscle is depleted and the post mortem lowering of pH ceases resulting in the ultimate pH of meat (Lawrie, 1998). The postmortem lower of muscle pH is one of the most important steps during the conversion of muscle to meat (Forrest et al., 1975).

Muscle pH is related to various meat quality parameters like tenderness, water holding capacity, cook loss, juiciness, and shelf life (Fletcher, 2002). Furthermore, the rate of postmortem pH decline has a significant impact on the meat quality. A rapid decline of pH postmortem may
result in conditions like Pale, Soft, Exudative (PSE) where the meat will have a lighter color and reduced water holding capacity (Owens et al., 2009). On the other hand, higher ultimate pH could impart a dark color to the meat due to increased water holding capacity (Forrest et al., 1975). Both the above conditions can result in reduced acceptability of the product.

The complete depletion of the sources of energy will result in the formation of permanent cross bridges between the actin and myosin filaments. As a result, muscle loses its elasticity and extensibility, and the phenomenon is known as rigor mortis or “stiffness of death” (Forrest et al., 1975). However, the stage is transient and the resolution of rigor mortis takes place due to the degradation of muscle structure. The postmortem decline of pH disrupts the membrane integrity of various cell organelles in muscle. This results in the release and activation of various calcium dependent proteolytic enzymes, mainly the calpain and catherspins (Forrest et al., 1975; Lawrie, 1998). These proteolytic enzymes will act on different muscle proteins, degrading the proteins resulting in resolution of rigor mortis. The process is known as “conditioning” or “ageing” and will help to improve the tenderness and flavor of the meat (Lawrie, 1998).

**E. MEAT QUALITY**

Meat quality is an important aspect which decides the purchase intent and also the repeatability of purchase by the consumer. The majority of the eating qualities of meat depend on the amount and properties of muscle fibers and the connective tissue present in it (Forrest et al., 1975; Lawrie, 1998). Appearance, tenderness, water holding capacity and juiciness, and flavor are some of the meat quality attributes (Lawrie, 1998; Fletcher, 2002). Appearance of the meat is the single quality attribute which determines the selection of raw meat products. Meanwhile, the appearance and tenderness of the cooked meat are important for the ultimate satisfaction and
repeatability of purchase. However, juiciness and flavor of the product mainly depends on the mode of preparation of the product (Fletcher, 2002).

**Color**

Skin, meat and bone color are important for various poultry products. But, for a product like boneless skinless broiler breast fillets, meat color is the critical factor. According to Fletcher (2002), the factors contributing to poultry meat color are myoglobin content, chemical state of the heme structure and meat pH. The myoglobin content is determined by the species, type of muscle, and age of the animal while the chemical state of myoglobin depends mainly on further processing and packing techniques used (Lawrie, 1998). Muscle pH is related to various events occurring prior to processing and the subsequent rigor development. A negative correlate has been reported between the breast meat lightness and meat pH (Allen and Goll, 2003). Besides these, there could be the occurrence of other visual defects due to bruises, hemorrhages and blood pooling which could result in reduced consumer acceptance (Fletcher, 2002).

**Tenderness**

Tenderness of meat is mainly contributed by three categories of proteins – sacroplasmic, connective tissue and myofibrilar proteins (Lawrie, 1998). The sacroplasmic proteins are water soluble, so their contribution to tenderness might be negligible. As the animal grows older, the collagen cross linking in the connective tissue increases and the meat will become tougher (Lawrie, 1998). Since the modern broilers are grown for less than 7 to 8 weeks of age, the age related toughness of connective tissue is not a major factor (Fletcher, 2002). Another factor which could dilute the toughening effect of connective tissue is the intramuscular fat or marbling (Lawrie, 1998). But, in poultry, the fat is deposited mainly under the skin and in the abdominal
fat pad rather than stored as intramuscular fat. So, the amount and contribution of intramuscular fat to poultry meat tenderness could be less. The major factor which determines the tenderness of poultry meat is mainly the contractile state of myofibrillar protein (Forrest et al., 1975). It has been reported that the properties of myofibrillar proteins depends on the live bird handling and stress prior to slaughter, processing techniques, the rate and severity of rigor mortis development and the physical handling of the carcass during rigor development (Fletcher, 2002).

**Water holding capacity and juiciness**

Water holding capacity is the ability of meat to retain its water under various conditions such as cutting, heating, grinding or pressing (Forrest et al., 1975). Water holding capacity of the raw and cooked meat can be accessed from drip loss and cook loss, respectively (Honikel, 1998). Water holding capacity mainly depends on the formation of lactic acid and the postmortem decline of pH. When the pH approaches the isoelectric pH (pH at which the positive and negative are equal) of the meat proteins, the amount of proteins that attracts water will be lower resulting in a reduced water holding capacity. This relationship between pH and water holding capacity is known as net charge effect (Forrest et al., 1975). Furthermore, availability of interstitial space within protein structure formed by the actin and myosin cross bridges can also affect the water holding capacity, known as steric effect (Forrest et al., 1975). Water holding capacity is related to various quality attributes like juiciness, tenderness and color of meat.

**Flavor**

Flavor is a complex attributes which depends on odor, taste, texture, temperature and pH of meat (Lawrie, 1998). Odor is the major factor which contributes to flavor which could be associated with volatile compounds in the meat. It has been suggested that the sulfur-containing
compounds and the carbonyls impart the “meaty” and “chickeny” aroma to poultry meat, respectively (Ramaswamy and Richards, 1982). The sulfur amino acids are the major source of sulfur containing compounds, while the carbonyls are formed from the lipids, fatty acids and amino acids in poultry meat (Ramaswamy and Richards, 1982). Nonetheless, taste panels are still widely used for the assessment of flavor (Lawrie, 1998).

F. MUSCULAR ABNORMALITIES IN BROILERS AND THEIR IMPACT ON MEAT QUALITY

The common reasons for the muscular abnormalities in different animals are congenital and/or inherited defects (like anatomic defects, muscular dystrophy, metabolic defects), degenerative conditions (ischemia, nutritional deficiencies, toxins, exertion or trauma), inflammation (bacterial, viral, parasitic or immune mediated), endocrine (hypothyroidism, hypercortisolism etc.), electrolytic imbalances (hypokalemia, hypernatremia etc.), neuromuscular (central, peripheral or neuromuscular junction) disorders and neoplastic conditions (Valentine and McGavin, 2012). However, muscular abnormalities in poultry will gain importance only when it is apparent as any meat quality defects or has any serious welfare impacts. Some of the muscular abnormalities in poultry which are significant in meat quality aspects are discussed below.

**Hereditary muscular dystrophy**

The hereditary muscular dystrophy is a condition seen in domestic fowl which are homozygous for an autosomal recessive gene (am) and the phenotypic expression varies due to the action of various modifying genes (Asmundson and Julian, 1956; Wilson et al., 1988). The affected birds will be characterized by broad shallow body, short thick limb bones, increased fat
content and hypertrophy of pectoral muscle which eventually undergo atrophy in older birds. The pectoral muscle is also characterized by gross white striations in the direction of muscle fiber which could be because of the bulging of the hypertrophied muscle fasciculi (Asmundson and Julian, 1956; Julian and Asmundson, 1963). The major properties of inherited dystrophic chicken include impaired righting ability when kept of their back, high serum creatine kinase levels, muscle with high levels of fat, cathepsins and mitochondrial enzyme and low levels of lactate dehydrogenase, signs of muscle fiber degeneration and enlarged sarcotubular system (Wilson et al., 1979). It has been suggested that the gross and histopathological lesions seen in the skeletal muscles of dystrophic domestic chicken are similar to those seen in muscular dystrophies of human beings (Julian and Asmundson, 1963). The histopathological studies of the condition revealed a wide variation in the fiber size, vacuolization and degeneration of the muscle fibers, mononuclear infiltration, fat deposition and increase in connective tissue (Jordan et al., 1959; Holliday et al., 1968; McMurtry et al., 1972; Julian, 1973). Furthermore, Barany et al. (1966) reported that there was a decrease in the sarcoplasmic proteins, myosin and actin in breast muscles of genetically dystrophic birds when compared to the normal chickens. Besides the effect on appearance of meat (due to white striations), it was also shown to affect the tenderness (Scholtyssek et al., 1967; Peterson and Lilyblade, 1969), fat content and fatty acid profile of the poultry meat (Jordan et al., 1959; Jordan et al., 1964; Chio et al., 1972). Even though majority of the studies on chicken muscular dystrophy have been conducted on dystrophic New Hampshire strains, there are also reports of similar condition in Cornish chickens (Wagner and Peterson, 1970). However, there are few reports of the occurrence of this condition in modern commercial broiler birds.
**Nutritional myopathy**

The deficiency of vitamin E, along with associated nutrients like selenium and sulfur containing amino acids, in the poultry diet may result in pathological condition such as encephalomalacia, exudative diathesis and nutritional myopathy in chicks, ducks and turkeys (Klasing, 2008). Among these, the nutritional myopathy is grossly characterized by white striations on breast and leg muscles while the histopathology reveals degeneration of muscle fibers with fragmentation, hyalinization, loss of striation, multiplication of sarcolemmal cells, infiltration by heterophils and clumping of fibers into eosinophilic masses (Dam et al., 1952; Machlin and Shalkop, 1956). Above studies also showed that the occurrence of the condition can almost completely be prevented by adequate levels of dl-α-tocopherol acetate (0.01%), l-cystine (0.24%) and dl-methionine (0.5%) in the diet ration. Scott and Desai (1964) reported that about 900-1025 µg/100 ml of total plasma tocopherol levels can completely prevent the occurrence of muscular dystrophy in chickens. However, Nesheim and Scott (1958) suggested that selenium alone cannot completely prevent muscular dystrophy even though it can reduce the occurrence of the condition caused by diets low in vitamin E and methionine. Desai (1968) showed that up to 1 ppm of selenium in poultry ration will improve the effectiveness of D-α-tocopherol acetate and L-cystine in prevention of nutritional muscular dystrophy. Carew Jr and Scott (1969) reported that the occurrence of nutritional myopathy can also depend on the growth rate of birds. They found that the limited feed intake and the resulting reduced growth rate prevented the development of myopathic changes in breast muscle even in birds fed with diets deficient in vitamin E and sulfur amino acids. Vitamin E along with the selenium dependent - glutathione peroxidase, catalase and superoxide dismutase is involved in eliminating the toxic intermediates formed during the free radical chain reaction (Herrera and Barbas, 2001). The deficiency of these
nutrients in the diet could have resulted in the disruption of the integrity of the cells resulting in
damage and myopathic changes. However, there are no recent reports of nutritional myopathy in
broiler which could be due to the use of adequate levels in vitamin E and associated nutrients in
the poultry diet. One the other hand, the current research is mainly focused on the effect of
vitamin E in preventing the development of poultry meat quality problems like PSE (Olivo et al.,
2001) and improving the oxidative stability of meat evaluated both by sensory and instrumental
methods (De Winne and Dirinck, 1996; Nam et al., 2003; Narciso-Gaytán et al., 2010; Zouari et
al., 2010).

**Deep pectoral myopathy (DPM)**

Deep pectoral myopathy (DPM) or Oregon disease or green muscle disease is a condition
affecting the supracoracoideus muscle (tenders) in broilers and turkeys. During contraction, the
supracoracoideus muscle, which is located in a compartment between the keel and the tough
inelastic fascia, may expand up to 20% of its weight. In some cases, there may not be enough
room for the muscle to expand and as a result the swollen muscle strangles itself leading to
ischemia (Siller, 1985; Mitchell, 1999; Bilgili and Hess, 2002). According to Wight and Siller
(1980), gross lesions of DPM include acute edema followed by green necrosis and replacement
of the caudal region by fibro-adipose tissue. Histopathological study of the green lesions showed
necrotic, anucleated muscle fibers surrounded by a fibrous capsule externally surrounded by
region of normal/regenerating muscle or fibro-adipose tissue. Electron microscopic examined
showed ischemic necrotic lesions with early loss of glycogen and disintegration of sarcoplasmic
reticulum, mitochondria, nuclei and Z-lines. Siller (1985) suggested that the condition could be a
result of the selection for improved muscle growth with a possible genetic component and the
selection based on the plasma creatine kinase levels could reduce the incidence. The incidence of
deep pectoral myopathy ranged from 0 to 1.88% (average 0.06%) in broilers (Kijowaski and Konstanczak, 2009). Besides the significant change in color (from pink to green), they also observed that the meat became more tough and fibrous towards the later stages of deep pectoral myopathy.

**Toxic myopathy**

Most frequently observed type of toxic myopathy in poultry is due to ionophores having a broad spectrum of activity but a narrow range of safety. The commonly used ionophore anticoccidial drugs (with optimal dosage) in poultry are monensin (100 to 125 µg/kg), salinomycin (60 to 75 µg/kg), lasalocid (75 to 125 µg/kg) and narasin (60 to 80 µg/kg), and when used at 20 to 50% overdose, toxicity can occur (Dowling, 1992). Monensin is the most studied ionophore for its adverse effects (Roder, 2011) and the myopathic lesions of monensin toxicity has been reported in broilers, laying hens, growing and breeder turkeys (Mitchell, 1999). Clinical signs of the ionophore toxicity are feed refusal, growth depression, inco-ordination, cream-colored diarrhea, dyspnea, leg weakness, muscular stiffness and/or weakness and sternal recumbency, while the post mortem examination may not show any specific gross lesions (Chalmers, 1981; VanderKop et al., 1989). However, the microscopic study done by (Chalmers, 1981) observed extensive myopathic lesions on major pectoral, supracoracoideus, cranial iliotibial, medial crural flexor, femoral adductor and gastrocnemius muscles. The histopathological lesions associated with ionophore toxicities in skeletal muscles are variable and usually include myofiber necrosis and fragmentation, eosinophilic granular masses of disrupted sarcoplasm, intermyofibrillar fatty infiltration, mitochondrial degeneration, infiltration of heterophils and macrophages into interstitium and sarcoplasm (Dowling, 1992). Sandercock and Mitchell (1999) reported that the selection for rapid growth and higher meat yield have made the
broilers more susceptible to monensin induced myopathy. It has been proposed that the toxicity of ionophores could result in the sodium-potassium imbalance across the sarcolemma which leads to an increased influx of calcium causing cellular damage mediated through the activation of various enzymes. Some of the studies suggested that the calcium could activate of intracellular phospholipase result in the damage of both the internal structure of the muscle fiber and also the plasma membrane (Jackson et al. 1984; Sandercock and Mitchell, 2003; Sandercock and Mitchell, 2004). The cell damage could also occur through the activation of calcium dependent proteases (Alderton and Steinhardt, 2000; Whitehead et al., 2006). The potentiation of the ionophore toxicity has been reported due to its interaction with other drugs like tiamulin, macrolide antibiotics, chloramphenicol, sulfonamides, etc (Dowling, 1992; Roder, 2011).

Lesions similar to ionophore toxicity can be seen in different species of animals due to toxicity from gossypol present in cottonseed meal and alsoother plants like Taxus spp., Nerium oleander (oleander), Cassia occidentalis (senna), Eupatorium rugosum (white snakeroot), vetch and Karwinskiahum boldtiana (coyotillo) (Roder, 2011). Henry et al. (2001) reported that higher levels (> 400mg/kg of feed) of gossypol in broiler diet can result in reduced feed intake, growth and feed efficiency along with liver damage and enlarged gall bladder. Furthermore, the toxicity in chicken due to C. occidentalis could produce gross and microscopic lesions in pectoral and leg muscles similar to those of ionophore toxicity (Mahon, 1999). Graziano et al. (1983) studied the effect of C.occidentalis extract in domestic chicken and found that the daily administration could result in weight loss, muscular weakness, degenerative histopathological lesions in skeletal and cardiac muscle. Electron microscopy study conducted by Cavaliere et al. (1997) revealed enlarged mitochondria with disrupted or excessively branched cristae in skeletal muscle from affected birds which is suggestive of mitochondrial myopathy. Besides this, the toxin from the C.
occidentalis can also result in alterations in lymphoid organs like spleen and bursa of fabricius (Silva et al., 2003).

**Pale Soft and Exudative (PSE) meat**

Pale, soft, exudative is a condition where the meat will have a pale color, soft consistency and poor water holding capacity and the causes of the condition may be genetic and/or environmental factors related to pre-slaughter stress (Owens et al., 2009). The incidence of the condition is mainly attributed to the rapid decline of pH in the post mortem period, while the temperature of the meat is high (Pietrzak et al., 1997). The occurrence of the condition could be due to the excess release of calcium ions stored in the sarcoplasmic reticulum of the skeletal muscle due to the defect in ryanodine receptors (Strasburg and Chiang, 2009). These calcium ions can stimulate the activity of various enzymes in muscle resulting in denaturation of the protein (Jackson et al., 1984; Alderton and Steinhardt, 2000; Sandercock and Mitchell, 2003; Mitchell and Sandercock, 2004; Whitehead et al., 2006). Pale, soft, exudative meat was initially reported in pork, but there are reports of similar condition in broiler chickens (Van Laack et al., 2000; Wilkins et al., 2000; Zhang and Barbut, 2005). Wilhelm et al. (2010) found that the broiler fillets with PSE had a lower pH, water holding capacity and shear force while a higher color (L* value), myofibrillar fragmentation index (MFI) and cook loss was also observed. The authors also reported the PSE meat showed electron microscopic lesions like shrinking and depolymerisation of myofilaments and Z-lines disorganization. A consumer study conducted by Garcia et al. (2010) did not show any significant difference between the normal and PSE chicken meat with respect to tenderness and flavor. However, the reduced water holding capacity and the aesthetic defect caused by the pale color of the PSE meat could cause economic loss to the producer. About 5 to 40% of the meat in the poultry industry is estimated to have PSE-type
characteristics which could result in an economic loss of $200 million per year (Owens et al., 2009).

G. IMPACT OF GROWTH RATE ON POULTRY MEAT QUALITY

Poultry meat has been gaining consumer preference in the meat market or last few decades. The per capita availability of poultry meat showed almost 118% increase in 2005 when compared to that in 1970 (Wells and Buzby, 2008). The increase demand for poultry meat among the consumers has put the producers under pressure for increased production. So, producers are continuously selecting the birds and feeding them with higher energy food to increase the output. As a result, from 1925 to 2010 the average weight of the bird increased from 2.5 to 5.8 lbs while the market age decreased from 112 to 47 d (National Chicken Council, 2011). More than 75% of the broilers processed in industry have a live weight of > 4.5 lbs (National Chicken Council, 2005). This is a great achievement from a production point of view, but the meat quality aspects need to be considered. Anthony (1998) reported that the high selection intensities, shorter generation intervals and reduced environmental influences resulted in reduced slaughter age, increased body weight, muscle yields and feed conversion efficiency at specific ages but reduced the meat quality properties like texture and flavor. Furthermore, the increased metabolic demands associated with the challenge for rapid growth or high egg production in poultry could increase the work-load on various organs or systems leading to evolution of various metabolic disorders. These conditions mostly affect the cardiovascular system resulting in flock mortality or the musculoskeletal system resulting in slow growth rate and lameness leading to economic loss and welfare concerns, respectively (Julian, 2005). Mahon (1999) suggested that the selection for enhanced growth could have serendipitously accompanied with the selection of inherent muscle fiber defects or insufficient capillary/fascial growth.
resulting in the associated myopathic lesions referred to as growth-induced myopathy. He opined that the most of the pathological conditions seen in modern poultry can be related to rapid growth rate (Mahon, 1999). According to Wang et al. (1999), the PSE condition caused by alteration in the ryanodine receptors could be result of selection for growth characteristics in turkeys. Focal myopathy, distinct from deep pectoral myopathy or inherited muscular dystrophy, is a muscle abnormality which may be related to the rapid growth in turkeys (Wilson et al., 1990). The authors proposed that the rapid growth of the muscle fibers may have outgrown the supporting systems which could have resulted in such a condition. Hoving-Bolink et al. (2000) reported that there was a lower capillary density in chickens with a higher percentage of breast muscle which could potentially reduce the oxygen supply to breast muscle. Velleman and Nestor (2003) studied the myosin heavy chain temporal and spatial localization during the turkey breast muscle development and found that the selection for growth rate may be associated with increased muscle damage. A study on the enzyme markers of muscle damage (CK, LDH and AST) and the histopathological analysis showed that birds with higher body weight had a greater myopathic changes in pectoralis major and biceps femoris muscle when compared to the birds with lower body weight (Macrae et al., 2006). They suggested that larger muscle fibers and the inadequate capillary supply in the higher body weight birds may have resulted in greater metabolic stress because of the large diffusion distances for oxygen, metabolites and waste products (Macrae et al., 2006). Meanwhile, Sandercock et al., (2009) reported that the genetic selection for high body weight and meat yield in broiler chickens resulted in significant aberration in muscle cell cation regulation leading to higher concentrations of sodium, potassium, magnesium and calcium per mg of ash in the muscle tissue. These changes could affect the muscle cell damage and with various meat quality problems. In addition, Abdullah and
Matarneh (2010) reported a higher shear force values for breast muscle from heavier broiler carcasses.

In contrast, there are also reports that the higher growth rate may not be always associated with adverse effect on meat quality. Berri et al. (2001) showed that selection for improved body composition on muscle and meat characteristics in broilers could result in the alteration of breast metabolism but had no negative influence on meat quality. However, Berri et al. (2007) reported that the increase in breast fillet weight and yield were accompanied by increased fiber cross section area, reduced glycolytic potential and reduced lactate content. This resulted in better further processing properties like greater pH at 15 min PM and ultimate pH, drip loss and L* values when compared to the muscle with smaller fiber cross section area. So it is difficult to confirm whether increased growth rate per se is associated with any meat quality problems in birds.

H. APPROACH TO THE CURRENT PROBLEM OF WHITE STRIPING

Current problem of white striping is gaining the attention of the poultry meat industry. Producers are greatly concerned about the impact of the white striping on poultry meat market. But, virtually no research has been conducted on the problem yet, which makes it difficult to deal the situation. The present study was intended to collect information about the condition which could help to conclude whether there is a benefit or risk associated with white striping in poultry meat. According to Valentine and McGavin (2012), the evaluation of a muscle condition is based on the associated clinical, serological, gross and microscopic findings. The general clinical signs of muscular abnormalities include abnormal gait, weakness, muscle spasm, atrophy/hypertrophy etc., while the serological study involves the estimation of enzymes indicating acute (CK) and
chronic (AST) muscle damage. The gross evaluation will be based on the changes in the size of the muscle, color and texture. A detailed microscopic study helps to appreciate the cytoarchitectural changes taking place in the tissue (Valentine and McGavin, 2012). The gross lesions of white striping characterized by the occurrence of white striations seem to be similar to some of the existing conditions in poultry like the hereditary muscular dystrophy or nutritional myopathy. However, the lack of any previous studies on the white striping makes it impossible to confirm the possible link, if any, with these conditions. Therefore, a multifaceted approach was adopted in the present study to evaluate the factors associated with occurrence of white striping and its impact on the meat quality aspects of the broiler breast fillets. Initially, there is a need to get the opinion of the consumers about the condition and whether it is anyway going to affect the purchase decision. The analysis of gross lesion (mainly the size of the fillet), serology and histopathology of the condition can help us to determine whether there is any pathological basis. In addition, the estimation of serological parameters could provide valuable information about blood markers so that we will be able to identify the chance of occurrence in live birds. The impact of white striping on meat quality especially the color and texture has to be determined since there is a chance that the white striations may be associated with increase in fat and/or connective tissue. Again, the same tissue changes can cause potential changes in proximate composition of the meat which can initiate health concerns among consumer. Estimation of white striping in different strains of broilers and also experiments with different levels of dietary vitamin E can help to identify/rule out factors associated with white striping. It will be also interesting to evaluate the effect of growth rate on the incidence of white striping since the selection for rapid growth rate is alleged to be reason for most of the muscular abnormalities (Mahon, 1999).
Figure 1: Phases of skeletal muscle response to muscle damage (modified from Natarajan et al., 2010)
I. REFERENCES


III. CONSUMER ACCEPTANCE OF VISUAL APPEARANCE OF BROILER BREAST MEAT WITH VARYING DEGREES OF WHITE STRIPING

A. ABSTRACT

White striping is a condition associated with heavier broiler breast fillets and is observed grossly as white striations seen parallel to the direction of the muscle fibers. The present study was intended to assess the consumer acceptance of broiler fillets with different degrees of white striping condition. High resolution digital images of fillets, representative of varying degrees of white striping, were shown to 75 consumers in a blind study. Individual images were presented using a completely balanced randomized design. There were four replicates of individual fillets within each white striping category (Normal - NORM, Moderate - MOD, and Severe - SEV) and one picture of tray pack (3 fillets) for each category. The consumers were asked to express their overall liking for appearance with a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely) and purchase intent using a 5-point scale (5 = definitely would buy; 1 = definitely would not buy). An open ended comments section was also included. The results showed that NORM fillets had a significantly higher hedonic score (6.9) than the MOD fillets (6.1) which was also significantly higher than the SEV fillets (4.5), indicating that as severity of white striping increased, the consumer acceptance decreased. From the distribution of the responses, 10.7, 22.4 and 56.7% of the consumers disliked the NORM, MOD, and SEV fillets, respectively. Furthermore, the average purchase intent score for the NORM fillets (3.6) was significantly higher than those with two degrees of white striping (2.4 and 2.5, respectively), suggesting that the consumers were more likely to buy NORM fillets. Over 50% of the consumers indicated that they would probably not or definitely not buy MOD or SEV fillets. The correspondence analysis of open ended comments revealed the major reasons for the dislike of the white striped meat was
that the fillets had a more fatty or marbled appearance. The results of the study suggest that the white striping does affect the consumer acceptance based on the appearance of the fillets.

**B. INTRODUCTION**

Poultry meat consumption has increased during the last few decades. Since 1970, the per capita consumption of chicken in US has been increased from 40 to 85 lbs, which is more than that of beef and pork (National Chicken Council, 2011c). Various factors like increased health concerns among the consumers, need for value added and convenient products and a change in relative prices may have resulted in increased consumer demand for poultry meat (Davis and Stewart, 2002). Kennedy et al. (2004) reported that consumers expressed a preference for buying raw over cooked chicken meat since they believe that the raw product gave them more control over the quality of the product. Results from a focus group study also showed that flesh appearance (indicating product freshness and healthfulness, including fat content) and convenience (ease in cooking the product) are the most important attributes favoring the choice of poultry meat (Kennedy et al., 2004). Furthermore, they found that the chicken fillets were highly preferred by the consumers because of the minimal preparation and cooking required, versatility to be used in various meals, and the good value since there is no waste. Now-a-days, about 88% of the broilers sold in US are in the form of cut up parts or further processed products (National Chicken Council, 2011a). Also, Leidahl (2006) reported that 65% of the consumers most often purchased boneless, skinless breast meat when purchasing chicken. However, to maintain the market, it is always important to produce quality products which meet the consumer demand.
Visual appearance is the primary and most important attribute available for the consumer to assess the quality of a meat product in a sealed package. Therefore, any condition negatively affecting the visual appearance of a product can influence the purchase decision potentially leading to economic loss. In fact, an annual revenue loss of about $1 billion is suffered as a result of the discounted sale of 15% of retail beef due to surface discoloration (Smith et al., 2000; Mancini and Hunt, 2005). White striping is a condition characterized grossly by the occurrence of white striations on the chicken fillets and thighs. There can be varying degrees of striations and these are seen parallel to the direction of the muscle fibers (Kuttappan et al., 2009). Since white striping can be easily identified on the surface of raw chicken breast fillets, the condition can affect the visual appearance of such an expensive product.

Some of the studies showed that the condition is associated with heavier birds (Bauermeister et al., 2009; Kuttappan et al., 2009). Kuttappan et al. (2009) estimated the percentage of occurrence of each degree of white striping in four different high yielding broiler strains and determined that percentages of fillets showing signs of MOD or SEV striping ranged from 41 to 72 (over 50% in 3 of the strains) suggesting the condition is widespread. Males also showed a greater incidence of striping compared to females (63 vs. 47%), likely related to body size/muscle mass differences. From a profit point of view, meat birds are continuously selected to have better growth rate and higher breast meat yield (Le Bihan-Duval et al., 1998; Berri et al., 2001). Since 1925, the average live weight of the broiler birds increased from 2.5 to 5.8 lbs (National Chicken Council, 2011b). According to National Chicken Council, (2005), about 77% of the broilers have > 4.21 lbs of live weight and are marketed as fresh, unprepared portions in the retail grocery or used for further processing. In this scenario, the result from the study conducted by Kuttappan et al. (2009) warns the possibility of increased occurrence of white
striping conditions in the industry. Even though, the striations become less distinguishable when the fillets are cooked and it may not cause any significant effect on various meat quality parameters (Bauermeister et al., 2009; Kuttappan et al., 2009), the visual appearance of raw meat could be affected. However, consumer acceptability is not known. Therefore, it is important to know the consumer responses regarding the effect of white striping on the visual appearance of the boneless, skinless breast fillets. The main objective of this study was to identify whether there is any difference in the consumer acceptance and purchase intent for broiler breast fillets with varying degrees of white striping.

C. MATERIALS AND METHODS

Samples

For the present study, fillets from broilers around the age of 6 - 8 weeks, processed at University of Arkansas (UA) Poultry Processing Pilot Plant, were evaluated over a period of one month to get representative samples. The birds were processed using commercial methods. Deboned breast fillets were visually screened and separated according to the degree of white striping: normal or no striping (NORM), moderate (MOD), or severe (SEV) (Kuttappan et al., 2009). Fillets classified as NORM did not show any distinct white lines. Fillets classified as MOD exhibited white lines, parallel to the muscle fibers, that were generally <1 mm thick but easily visible on the fillet surface. Fillets classified as SEV exhibited white lines, parallel to the muscle fibers that were generally > 1 mm thick and very visible on the fillet surface. Examples are shown in Figure 1. Fillets were aged at 4°C for 24h in individually packed zip-sealed bags. Various studies have already been successfully conducted using the digital color photographs to evaluate consumer choice for beef and pork meat (Ngapo et al., 2003; Ngapo et al., 2004;
Dransfield et al., 2005; Fortomaris et al., 2006; Ngapo et al., 2007; Brugiapaglia and Destefanis, 2009). The present study used photographs to evaluate the consumer acceptance of raw breast fillets. At 24 h PM, high resolution pictures of the most appropriate samples under each category of white striping were taken using digital camera (Nikon D300, Nikon Inc., Melville, NY). The pictures best representing each category were selected for the consumer study. Special care was taken to select pictures that were uniform in appearance in terms of size, shape and color so that the actual striping condition was the primary factor that varied among pictures. Additionally, a group of 3 fillets per category (of similar size) were placed in a tray pack container to simulate a retail product. Tray packs were not wrapped in packaging film so that glare could be minimized. Pictures of the tray packs were then made. There were four replicates of individual fillets within each white striping category and one picture of tray pack (with three fillets) for each category that were used for sensory analysis.

**Sensory analysis**

Consumer acceptance of the fillet pictures were evaluated using 75 subjects selected from a database. The only criterion for selection was that the participants should habitually buy chicken fillets at least once in a month. Subjects participating in the panel were categorized into six age groups: 18 to 24 (n = 10), 25 to 35 (n = 16), 36 to 45 (n = 16), 46 to 54 (n = 10), 55 to 65 (n = 18) and over 65 (n = 5). The consumer study was conducted under the experimental conditions at the University of Arkansas Sensory Service Center (Fayetteville, AR). All the pictures were shown to the consumers on a computer screen (image size: approximately 25 cm X 19 cm) in a completely balanced randomized design. Each consumer had to evaluate all of the 15 (12 individuals and 3 tray packs) pictures (example of the pictures given in Appendix 1) in a sequential monadic pattern (each image evaluated one after the other). In order to minimize the
order effect, the order of presentation was randomized using a 12 - picture Williams design (all
the individual fillet pictures), followed by a 3-picture Williams design (all the tray pack pictures)
(Williams, 1949; Macfie et al., 1989). There was no warm up sample or “dummy” sample used
in the study as it could result in increased consumer fatigue. For the individual fillets, the
consumers were allowed to express their overall liking for the visual appearance of each fillet
picture on a 9-point (1 = “dislike extremely”, 9 = “like extremely”) hedonic scale (Peryam and
Pilgrim, 1957) and were also asked an open ended question to explain what they liked or disliked
about each fillet. The purchase intent of the tray pack pictures were assessed using a 5-point
scale (1 = “definitely would not buy”, 5 = “definitely would buy”). A sample questionnaire is
given in Appendix 2.

**Image analysis**

Image analysis is a technique widely used to evaluate meat and fish samples based on the
difference in color intensities in the images (Basset et al., 2000; Del Moral et al., 2007; Stien et
al., 2007). Image analysis could be a useful tool in the present study since white striping results
in color differences (area of white striations and flesh with pink color) on fillet surface. The use
of image analysis in this study was intended to estimate the area of white color on the fillet
picture so that it could aid in categorizing the different degrees of white striping. Image-Pro Plus
Software (Version 6.2; Media Cybernetics Inc., Bethesda, MD) was used to do the image
analysis of all the individual fillet pictures in order to quantify the degree of white striping. For
image analysis, the border of each fillet was first demarcated in each individual picture. Then,
using a pointer tool, an area with white color representative of the white striations was selected.
The automated program associated with the software highlighted all the pixels in the fillet image
with the same color intensity. Finally, the white area in each fillet picture was expressed as the
percentage of the total of the respective fillet. For each individual fillet picture, six replicate values (i.e., based on different areas of white color located on fillet, selected using pointer tool) of white area were obtained and averaged. The results were compared with the consumer responses.

**Data analysis**

Analysis of variance (ANOVA) was used to evaluate the overall liking, purchase intent scores and the image analysis results of the fillet pictures. In both cases, ANOVA was performed considering the degrees of white striping (NORM, MOD and SEV) as fixed source of variation (JMP, Version 8; SAS Institute Inc., Cary, NC). The mean scores were estimated for each degree of white striping and significant differences were calculated using Tukey’s test ($P < 0.05$). Chi square test ($P < 0.05$) used to analyze the frequency of occurrence of responses.

The open ended comments from the consumers regarding the reason for like or dislike of the individual fillets pictures were analyzed in-depth based on previous studies (ten Kleij and Musters, 2003; Ares et al., 2010). Initially, all the open-ended comments from the consumers were screened to identify recurring terms. The terms having similar meaning were manually grouped into different categories for each degree of white striping. Those categories containing terms used by more than 10% of the consumers were considered in this study. There were comments containing terms related to more than one category. Those comments were split up based on the different terms and were considered under the respective categories. Only a few comments (<1% of the total responses) mentioned a neutral opinion (e.g., neither like nor dislike, no comments) which were not included in this study. The number of consumers who mentioned the terms in each category was counted in order to estimate the frequency for each
category. Correspondence analysis was used to visualize the relationship between the replicates of fillets with various degrees of white striping and the terms used to explain them (JMP, Version 8; SAS Institute Inc., Cary, NC). Correspondence analysis is a generalized principal component analysis used for qualitative data (Abdi and Williams, 2010). It is used to analyze the correspondence between the rows and columns of a two dimensional contingency table. Both the row and column variables will be represented on the same geometrical space which allows the visual interpretation of the data (ten Kleij and Musters, 2003; Ares et al., 2010). Chi square test ($P < 0.05$) was used to evaluate the differences in the number of responses in each category (after combining the replicates) for each degree of white striping. The combining or collapsing of the replicates in each category was not done in correspondence analysis as it may result in overfitting.

D. RESULTS AND DISCUSSION

The results from the mean consumer overall liking and the percent of white area were as shown in Table 1. The fillets classified as NORM had a significantly higher hedonic mean score compared to the fillets in either MOD or SEV categories which were also significantly different from each other. The SEV fillets had the lowest acceptability (lowest hedonic mean, 4.5) and would actually be considered as dislike by consumers. This indicates that there was a significant decrease in the consumer acceptance for the chicken fillets when the severity of the white striations increased. Table 2 shows the difference in frequencies of the various responses in the 9-point hedonic for the three degrees of white striping. In the present study, NORM fillets had a significantly higher percent of “like very much” and “like extremely” (hedonic scale rating ≥ 8) responses compared to MOD and SEV fillets. The SEV fillets had a significantly higher percent of dislike responses compared to NORM (hedonic scale ≤ 4) or MOD (hedonic scale ≤ 3). In
fact, over 50% of the consumers reported that they disliked the visual appearance of those fillets categorized as SEV whereas the percentages of dislike for MOD and NORM were around 22% and 11%, respectively.

Image analysis was conducted to quantify the degree of striping. There was a significant increase in the percentage of white area (calculated from image analysis) as the degree (visually categorized) of white striping increased, as expected; indicating that image analysis could be a useful tool in classifying fillets (Table 1). Furthermore, there was a high negative correlation between the hedonic score and the percentage of white area in the fillet pictures (Figure 2). This again confirms that the different degrees of white striping caused a decrease in consumer acceptance. However, the main limitation for the image analysis was that it over-estimated the degree of white striping. The white area calculated by the software includes white color found all over the fillets (e.g., connective tissue present on the surface or glare from the camera flash) and not just the white striations. For example, the white area calculated in the NORM fillets was 8.42% when no white striations were present. This percentage could be considered a baseline and would be expected to be accounted for in the other categories as well, with the increase in white area mainly attributed to the increase in white striations rather than background color. While the estimation of white striping in fillets may be over-estimated, the image analysis was still highly correlated to degree of white striping. Further work would be needed to optimize the quantification of white striping.

When assessing purchase intent (Table 1), consumers rated the NORM tray pack picture more closely to “probably would buy” (3.6 on scale) which was a significantly higher rating than tray packs containing either MOD or SEV fillets. Ratings of the MOD and SEV tray packs were not significantly different and they were closer to “probably would not buy” on the scale (2.4 and
2.5, respectively). Because consumer acceptance (according to 9 point hedonic scale) decreased as severity of striping increased, the significant decrease in purchase intent is not surprising. However, the lack of difference between the MOD and SEV samples was interesting. The frequency of the purchase intent scores for the three degrees of white striping (as shown in Table 2) again confirms that the consumers were significantly less willing to buy samples with any white striations on the surface. More than 50% of the consumers responded that they would not buy (rating of ≤ 2 on purchase intent scale) either the MOD and SEV samples compared to 16% for the NORM fillets. These results suggest that the consumers were able to detect the occurrence of white striping and perceived it as a negative attribute. These results also indicate that the majority of consumers were not willing to buy a fillet with any degree of white striping, regardless of the severity of the condition.

It is important to identify reasons for consumer likes or dislikes for the product; therefore, open ended comments were included in the sensory panel. Open-ended responses from the present study were evaluated and analyzed using correspondence analysis in order to use the valuable information from the qualitative data for complementing the quantitative findings (ten Kleij and Musters, 2003; Ares et al., 2010). Manual evaluation of all the open ended questions revealed the major categories of words were related to fat, color, freshness, visual texture, white lines, size and uniformity and the look of the fillets (Table 3). The category “look good”, which refers to the general look of the fillet, is a non-specific category where the consumer did not give specific reason for like or dislike. For each category, there were terms used to express both the positive and negative aspects of the respective categories as shown in the examples. The Chi-square analysis with the number of responses under each category showed that there were some significant differences with respect to the degree of white striping. Generally, the SEV samples
had a greater number of negative comments whereas the NORM samples had a greater number of positive comments.

The plot from the correspondence analysis (Figure 3) provides a better way to determine the relationship between the terms used and the replicates of fillets with three degrees of white striping. The two components, C1 and C2, explained 67.1% and 15.9% of the variability in the data, respectively. The overall liking score was projected to the correspondence analysis plot in order to determine the factors which drive consumer liking. The projection was done based on the correlation between the two components (C1, C2) of correspondence analysis and the overall liking (OL) hedonic scores for the respective fillets \[ r (C1, OL) = 0.96; \ r (C2, OL) = 0.15 \]. The direction of the vector represents the direction of increasing consumer liking. In the present study, NORM and the SEV samples were seen on the opposite ends of the vector with NORM samples towards the increased liking end. MOD samples were seen spread out between the NORM and SEV. This pattern is similar with the pattern of the mean hedonic score for the NORM, MOD and SEV samples. Furthermore, the NORM samples had the highest percent of like responses and the lowest percent of dislike responses (Table 2). This suggests that the terms associated with NORM samples can be the factors driving the consumer liking. Meanwhile, the terms associated with SEV (with the lowest percent of like responses and highest percent of dislike responses) samples can be the factors driving the consumer dislike.

When the points on the correspondence analysis plot were evaluated with respect to the vector, analysis of the open-ended comments showed that the absence of white lines and fillet appearing low fat were the major reasons for the consumer liking broiler breast fillets in this study. Meanwhile, high fat (as a comment) and presence of white lines were the two major reasons for dislike. The comments, high fat and white lines, are likely used synonymously in
this study as no other fat (seam fat) was present in the pictures. The white lines may have given
the impression of the intramuscular fat as seen in beef or pork meat. Grunert (1997) suggested
that the quality evaluation of meat products by the consumer is mainly based on appearance
attributes such as the fat content and color. Consumers perceive fat on the negative aspect
associated with it, while the positive effect of fat on the flavor and tenderness are not considered
important (Grunert, 1997; Grunert et al., 2004). Fernandez et al. (1999) showed that the increase
in the visible fat (due to increase in intramuscular fat) in pork meat results in a reduction in the
consumer willingness to purchase and eat the meat. Also, Resurreccion (2003) suggested that the
increased health concerns among the US consumers, regarding the fat content in the meat
products and health problems (e.g., obesity, high blood pressure and heart diseases) may have
resulted in the decline in consumption of veal, beef and lamb. In this situation, the perception of
increased visible fat (in the form of white striping) on fillets surface can result in serious dislike
among the consumers leading to the high risk of poultry meat rejection. The consumers liked the
color of the NORM samples, which can be indirectly due to the absence of white striations
interfering with the normal color of the meat samples. The consumers responded that the SEV
sample had a bad visual texture, which might imply that it seems to be tougher. The white
striations may have given the impression of connective tissue to these consumers which may
have resulted in grading it as tough. Lastly, the white striations appeared to some consumers
possibly as a sign of spoilage which resulted in the feel of reduced freshness in SEV samples.

Interestingly, the non-specific “good look” terms (especially the positive terms), were
seen associated with the explaining the liking of NORM samples. The Chi-square test of the
counts of the responses also shows that there was a significant decrease in the number of “good
look” terms as the degree of white striping increased. However, this trend was not seen in the
case of “no good look” terms and the results from both the Chi-square test and the correspondence analysis showed that it is not influencing the decision of like or dislike. The possible reason for this can be that the respondents are inclined to give more specific comments on their dislikes than on their likes. In other words, they find it easier to give a specific reason or they are more motivated when they dislike a product (ten Kleij and Musters, 2003). Similarly, terms related to “no good look”, “good size & uniformity” and “bad size & uniformity” were present near to the point of origin of both axes which indicates that these terms were almost equally used for all the three degrees of white striping and did not contribute much to the like or dislike of the product. The finding was in accordance with the results from the Chi-square test of the counts (Table 3) where there was no significant difference between the three degrees of white striping in case of these three categories (no good look, good size and uniformity and bad size and uniformity). Thus, the analysis of the open-ended comments could provide important information regarding the consumer perception. However, the main disadvantage is that the information obtained is subjective and the data is analyzed with the response counts, not based on any standardized intensity scales (Dooley et al., 2010).

E. CONCLUSION

Based on the results of this study, it can be concluded that the presence of white striping (and increasing severity) decreases consumer acceptability of broiler breast fillets, based on appearance. More than 50% of the consumers reported that they would probably/definitely not buy the fillets with any degree of white striping. One of the major reasons for the consumer dislike was that the fillets with severe degree of white striping looked fatty (i.e., high fat). Since low fat content is an important attribute contributing to the increased consumer demand for chicken meat, the occurrence of white striping can result in serious rejection of the product. The
present study also showed that image analysis can be used as a valuable technique for qualifying the degree of white striping. However, the limitations of the technique have to be rectified by further research in order to use it as a potent automated processing inline tool to separate fillets with different degrees of white striping. Furthermore, this study confirms the use of photographs as an effective tool for the sensory evaluation of the visual appearance of poultry meat, especially the raw products. An added advantage of using pictures is that the same study can be repeated with another set of consumers with little variation in the samples and can get comparable results.
F. ACKNOWLEDGEMENT

The authors are appreciative for the support of Cobb-Vantress, Inc. (Siloam Springs, AR) and the University of Arkansas, Division of Agriculture (Fayetteville, AR) throughout this project. The authors would also like to thank Fred Miller and Sara Landis for their assistance in photography for this project.
G. REFERENCES


Table 1: Consumer hedonic, percentage of white area, and purchase intent score means for three degrees of white striping

<table>
<thead>
<tr>
<th>Degree of white striping</th>
<th>Individual fillet pictures</th>
<th>Tray Pack Pictures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hedonic score&lt;sup&gt;1&lt;/sup&gt;</td>
<td>White area (%)&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>NORM</td>
<td>6.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.42&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MOD</td>
<td>6.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28.55&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SEV</td>
<td>4.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>54.80&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Pooled SEM</td>
<td>0.1</td>
<td>3.77</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Means in each column with different letters are significantly different ($P < 0.05$)

<sup>1</sup>Hedonic score 1 = dislike extremely, 9 = like extremely; n = 300 per mean

<sup>2</sup>Image analysis, n = 4 per mean

<sup>3</sup>Purchase Intent Score: 1 = definitely would not buy, 5 = definitely would buy; n = 75 per mean
Table 2: Frequency (%) of consumer responses for hedonic and purchase intent scales for three degrees of white striping

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Degree of white striping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NORM</td>
</tr>
<tr>
<td><strong>9-point hedonic scale</strong></td>
<td></td>
</tr>
<tr>
<td>Dislike extremely (1)</td>
<td>1^b</td>
</tr>
<tr>
<td>Dislike very much (2)</td>
<td>1^b</td>
</tr>
<tr>
<td>Dislike moderately (3)</td>
<td>3^b</td>
</tr>
<tr>
<td>Dislike slightly (4)</td>
<td>5.67^b</td>
</tr>
<tr>
<td>Neither dislike or like (5)</td>
<td>6.33^a</td>
</tr>
<tr>
<td>Like slightly (6)</td>
<td>10.67^a</td>
</tr>
<tr>
<td>Like moderately (7)</td>
<td>26^{ab}</td>
</tr>
<tr>
<td>Like very much (8)</td>
<td>36.67^a</td>
</tr>
<tr>
<td>Like extremely (9)</td>
<td>9.67^a</td>
</tr>
<tr>
<td><strong>5-point purchase intent scale</strong></td>
<td></td>
</tr>
<tr>
<td>Definitely would not buy (1)</td>
<td>4.05^b</td>
</tr>
<tr>
<td>Probably would not buy (2)</td>
<td>12.16^b</td>
</tr>
<tr>
<td>Maybe/maybe not (3)</td>
<td>20.27^b</td>
</tr>
<tr>
<td>Probably would buy (4)</td>
<td>43.24^a</td>
</tr>
<tr>
<td>Definitely would buy (5)</td>
<td>20.27^a</td>
</tr>
</tbody>
</table>

^1 Values given in parenthesis are the numerical score for the respective attribute

^a-c Percentages in each row with different letters are significantly different (P < 0.05)
Table 3: Categories of terms, examples of comments and the frequencies of occurrence for each degree of white striping

<table>
<thead>
<tr>
<th>Categories</th>
<th>Examples of open ended comments</th>
<th>Degree of white striping</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NORM</td>
</tr>
<tr>
<td><strong>Fat</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (+)*</td>
<td>Leanness, not much fat, less fat</td>
<td>110^a</td>
</tr>
<tr>
<td>High (-)*</td>
<td>Extra fat, marbling, too much fat</td>
<td>5^c</td>
</tr>
<tr>
<td><strong>Color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good color (+)</td>
<td>Nice color, healthy color, fair color</td>
<td>84^a</td>
</tr>
<tr>
<td>Bad color (-)</td>
<td>Color is not good, dislike the discoloration</td>
<td>14^b</td>
</tr>
<tr>
<td><strong>Looks fresh</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fresh look (+)</td>
<td>Fresh chicken breast, like the freshness</td>
<td>42^a</td>
</tr>
<tr>
<td>No fresh look (-)</td>
<td>Looks old, doesn't look as fresh</td>
<td>11^b</td>
</tr>
<tr>
<td><strong>Visual texture</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good texture (+)</td>
<td>More tender looking, looks very tender</td>
<td>26^a</td>
</tr>
<tr>
<td>Bad texture (-)</td>
<td>Tough, not tender, do not like the texture</td>
<td>10^b</td>
</tr>
<tr>
<td><strong>White lines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No white lines (+)</td>
<td>No striations, did not have the odd lines</td>
<td>18^a</td>
</tr>
<tr>
<td>White lines (-)</td>
<td>Too many striations, 'striped' look</td>
<td>0^b</td>
</tr>
<tr>
<td><strong>Size and uniformity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good size &amp; uniformity (+)</td>
<td>Good sized, uniformed chicken breast</td>
<td>36^a</td>
</tr>
<tr>
<td>Bad size &amp; uniformity (-)</td>
<td>Odd shaped, I don’t like the size</td>
<td>15^a</td>
</tr>
<tr>
<td><strong>Looks good</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Good look (+)</td>
<td>Looked good, look pretty good</td>
<td>66^a</td>
</tr>
<tr>
<td>Bad look (-)</td>
<td>Whole appearance was not that good, does not look normal</td>
<td>20^a</td>
</tr>
</tbody>
</table>

^a-c Counts in each row with different letters are significantly different (P < 0.05)
* “Positive (+)” and “negative (-)” are terms based on the consumers’ like or dislike
Figure 1: White striping classification of broiler breast meat: A) normal (NORM) breast fillet (no striping), B) breast fillet exhibiting moderate (MOD) degree of white striping, and C) breast fillet exhibiting severe (SEV) degree of white striping.
Figure 2: Correlation between image analysis and the hedonic scores for the individual fillet images. (Hedonic score = -0.051*White area% + 7.415; $R^2=0.921\ P < 0.0001$)
Figure 3: Correspondence analysis of the terms used in the open-ended responses for three degree of white striping ( ■ - replicates with three degrees of white striping; ◆ - categories of terms used in open ended comments)
H. APPENDIX

1. Examples of pictures presented to human subjects for the sensory evaluation of the visual appearance of broiler breast fillets with different degrees of white striping

Example 1 (single fillet)

Example 2 (single fillet)
Example 3 (tray pack of fillets)
2. **Ballot used for the consumer evaluation of the visual appearance of broiler breast fillets with different degrees of white striping**

Instructions shown on computer screen before the survey is given.

Your participation in this study is completely voluntary. If you choose to participate, your identity will be kept confidential. You will be assigned a panelist number (code) and individual responses will be analyzed using panelist number rather than your name. You are free to withdraw from this study at any time with no penalty to you.

In this study, you will be shown 15 digital pictures. Following each picture, you will be asked a series of questions. The questions will be in the form of rating scales or open ended comments where remarks can be made about each picture. Three demographic questions will also be asked.

**If you choose to participate, please continue with the following screens that will include pictures and questions related to the pictures.**

This research has been reviewed by the Institutional Review Board at the University of Arkansas. For research-related problems or questions regarding human subjects’ rights, please contact Dr. Rosemary Ruff, Director of Research and Sponsored Programs, at (479) 575-3845. You may also contact Dr. Casey Owens, principal investigator, at (479) 575-4281 should you have any questions regarding the research.

Your participation in this study is appreciated very much.
Question # 1 - Sample ______

You should have been shown a picture of fillet. If you were shown fillet, please select one from the options below. If not, please press the alarm.

☐ Single Chicken Breast
☐ Tray Pack of Three Breasts

Question # 2 - Sample ______
Which statement best describes your impression of the APPEARANCE of this product?

Appearance

<table>
<thead>
<tr>
<th>Dislike Extremely</th>
<th>Dislike Very Much</th>
<th>Dislike Moderately</th>
<th>Dislike Slightly</th>
<th>Neither Like nor Dislike</th>
<th>Like Slightly</th>
<th>Like Moderately</th>
<th>Like Very Much</th>
<th>Like Extremely</th>
</tr>
</thead>
</table>

Question # 3 - Sample ______

What do you LIKE and/or DISLIKE about the sample image you were just shown?

-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------
-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

Question # 4 - Sample ______

When only considering the appearance of the chicken in the tray pack, how would you describe your purchase intention compared to your normal chicken purchase?

Purchase Intent

<table>
<thead>
<tr>
<th>Definitely Would Not Buy</th>
<th>Probably Would Not Buy</th>
<th>May or May Not Buy</th>
<th>Probably Would Buy</th>
<th>Definitely Would Buy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Question # 5.
What is your gender?
☐ Male  ☐ Female

Question # 6.
To which age group do you belong?
☐ Under 18 years old
☐ 18-24 years old
☐ 25-35 years old
☐ 36-45 years old
☐ 46-54 years old
☐ 55-65 years old
☐ Over 65 years old

Question # 7.
Which category best represents your annual household income?
☐ Under $15,000 per year
☐ $15,000 to $19,999 per year
☐ $20,000 to $29,999 per year
☐ $30,000 to $39,999 per year
☐ $40,000 to $49,999 per year
☐ $50,000 to $59,999 per year
☐ $60,000 to $69,999 per year
☐ $70,000 to $79,999 per year
☐ $80,000 to $89,999 per year
☐ $90,000 to $99,999 per year
☐ more than $100,000 per year
IV. ESTIMATION OF FACTORS ASSOCIATED WITH THE OCCURRENCE OF WHITE STRIPING IN BROILER BREAST FILLETS

A. ABSTRACT

Broiler breast fillets are sometimes characterized grossly by white parallel striations in the direction of the muscle fibers and the condition is referred to as white striping. Depending on the severity of white striping, fillets can be classified as normal (NORM), moderate (MOD) or severe (SEV). The present study was intended to determine the factors associated with the occurrence of white striping in broiler breast fillets. Broiler birds (59-63d), of four different commercial high yielding strains (both males and females) fed with industrial type (IF) or phase-feeding (PF) regimens, were processed and Ready-To-Cook (RTC) carcass weight was recorded. The carcasses were deboned at either 4 or 6 h post mortem (PM). Fillets were scored for the degree of white striping at 24h PM and dimensions of fillets (length, width, cranial thickness and caudal thickness), pH24 and color (L*24, a*24 and b*24 values), cook loss, and Meullenet-Owens Razor Shear Energy (MORSE) values were determined. About 55.8% of the birds used in the study showed some degree of white striping with MOD and SEV categories as 47.5 and 8.3%, respectively. Higher degrees of white striping were significantly \( (P < 0.05) \) related to higher cranial fillet thickness and RTC weights. The occurrence of SEV degrees of white striping was accompanied with increased b* values or yellowness of the meat. There could be difference with respect to growth differences in strains, but feeding regimens as well as chill hour during processing did not influence the incidence of the condition. In addition, the degree of white striping did not show any significant \( (P > 0.05) \) relationship between various meat quality parameters like pH24, L*24, a*24, cook loss and tenderness. In conclusion, the results of this study
suggest that there is a greater chance of higher degrees of white striping to be seen associated with heavier birds, but the condition is not related to any major changes in meat quality.

B. INTRODUCTION

The quality of a product is a multifaceted concept. Various factors involved in overall quality could be organoleptic quality, nutritional quality, safety, psycho-social acceptance and the properties which support further processing (Rémignon and Bihan-Duval, 2003). Poultry meat has been widely accepted for its quality which resulted in a tremendous development in the poultry industry for the last few decades. In order to remain successful, the growing consumer demands, both quality and quantity aspects, have to be met. Therefore, there is a pressure to increase the production efficiency, and at the same time, provide the consumers with good quality meat. Recently, some producers have noticed the occurrence of white striping, which could be a potential threat to the consumer preference of the product (Kuttappan et al., 2012a). It occurs as white striations, seen parallel to the direction of muscle fibers. The condition is mainly seen on raw breast fillets but can also occur on tenders and some thigh muscles. Furthermore, histopathological studies revealed that the condition shows muscle damage characterized by degeneration of muscle fibers along with increase in fat (lipidosis) and connective (fibrosis) tissues (Kuttappan et al., 2009; 2011). Both the lipidosis and fibrosis could affect the various meat quality parameters, mainly the color of the raw meat and tenderness of the cooked product. Therefore, it is important to know whether the different degrees of white striping have any impact on the quality of the breast fillets.

Some of the researchers suggest that the intensive selection of birds for increased live weight and breast yields may result in reduced meat quality (Wilson et al., 1990; Sosnicki and
Wilson, 1991; Pietrzak et al., 1997; Dransfield and Sosnicki, 1999). Velleman and Nestor (2003) studied the growth rate on myosin heavy chain temporal and spatial localization in turkey breast muscle and found that growth selection resulted in muscle fiber changes which may result in muscle damage. Even the genetic increase in the egg production may also be associated with changes in muscle morphology, however to a lesser extent than the selection for increased growth rate (Velleman et al., 2007). Since white striping is an emerging issue, it is important to evaluate whether there is any association between the occurrence of white striping and the size of the bird/fillet. If that is the case, the occurrence of the condition may presumably be influenced by the strain and/or the gender of the bird as these are some of the factors which could influence the carcass yield (Orr et al., 1984; Bilgili et al., 1992; Young et al., 2001; Mehaffey et al., 2006). Moreover, the feeding regimens like Phase feeding (PF), which could reduce the production cost as well as improve the uniformity of the fillets (Warren and Emmert, 2000; Pope et al., 2002; Brewer et al., 2006a; Brewer et al., 2006b), might be a useful tool to manipulate the occurrence of the condition. Therefore, the purpose of this study was to estimate the association of various factors (strain, gender, feeding regimen, various carcass and meat quality parameters) with the three (NORM, MOD and SEV) degrees of white striping.

C. MATERIALS AND METHODS

**Processing of Birds**

A total of 739 birds, from the study conducted by Brewer et al. (2012), were randomly screened to determine the occurrence of three degrees of white striping. The distribution of the birds used in the present study was as given in Table 1.The birds were grown in 4 X 2 X 2 factorial design in six replicates. There were four strains of commercial broiler birds, balanced
with respect to gender. Among the strains, A, B and D were bred to be high yielding broilers while strain C was bred to be moderate yielding classic type broiler. These birds were grown on either of the two diet treatments, industry-type feeding (IF) regimen or a phase feeding (PF) regimen (Brewer et al., 2012). Birds were processed on 59, 61 and 63 d. About 10 h before slaughter, feed was withdrawn but the birds were given an ad libitum supply of water. A commercial-style processing in-line system was used where the birds were electrically stunned, manually slaughtered by severing the left carotid artery and jugular vein, bled out, soft scalded and defeathered. The carcasses were then manually eviscerated, prechilled at 12°C for 15 min followed by chilling for 90 min at 1°C in immersion chilling tanks. While prechilling and chilling, the carcasses were manually agitated frequently in order to prevent the thermal layer in the tank and to enhance the chilling efficiency. The carcasses were taken out of the tanks, packed in ice and aged at 4°C until deboning at 4 or 6 h post mortem (PM). Ready-to-cook (RTC) weight of each carcass was measured before deboning. The pectoralis major muscle was removed from each carcass by two trained people in order to avoid any alterations in fillet dimensions and other meat quality parameters due to the deboning procedures. The butterfly fillet from each bird was placed in zip-lock bags and stored at 4°C for 24h PM.

**Carcass and meat quality parameters**

At 24h PM, the fillets were scored as NORM, MOD or SEV degrees depending up on the severity of white striping (Kuttappan et al., 2012a). NORMs are the ones which do not show any distinct white lines while the SEV will have thick white striations (> 1mm) which cover a greater area of the surface of the fillet. The fillets showing striations intermediate to NORM and SEV are classified as MOD, with thin white striations (< 1mm) on the surface. After scoring, each butterfly fillet was halved into left and right. The right fillets were used for measuring pH24 and
color, while fillet dimensions were estimated on the left fillets. Later, left fillets were vacuum packed and stored in the freezer (at -29°C) until the cook loss was determined. Muscle pH$_{24}$ was measured using a Testo spear tip probe and meter (Model Testo 205; Testo Inc. Sparta, NJ). The L*$_{24}$, a*$_{24}$ and b*$_{24}$ color values were determined as an average of 3 different sites on the dorsal (bone side) of the fillet using a Minolta colorimeter (CR-300; Konica Minolta, Ramsey, NJ). In order to determine the fillet dimensions, length (at the longest point), width (at the widest point), cranial thickness ($H_1$ is the height at the thickest portion) and caudal thickness ($H_2$ is the height at 2.5cm from the bottom of the fillet) were measured using calipers (Mehaffey et al., 2006). The fillets were vacuum packed and stored at -20°C until the cook loss and tenderness were estimated. Before cooking, the fillets were taken out of the freezer and thawed at 4°C for 24h. All fillets were cooked separately, on raised wire racks in covered aluminum-lined pans in an air convection oven to an internal end-point temperature of 76 °C. The difference between fillet weights before and after cooking was taken and cooking loss was expressed as percent with respect to the initial weight. After cooking, the fillets were cooled to room temperature, individually wrapped in aluminum foil and stored overnight at 4°C, to be used for the determination of tenderness. Meullenet-Owens Razor Shear (MORS) technique (Cavitt et al., 2004) was used to determine tenderness of the cooked samples and the results are reported in terms of shear energy or MORSE (Nmm). The method uses a texture analyzer (model TAX-T2, Texture Technologies, Scarsdale, NY) with a 5-kg load cell using a razor blade probe. Four shear readings, at different locations, were done perpendicular to the muscle fibers on each fillet and the mean was taken. The crosshead speed was 5mm/s along with a sample shear depth of 20 mm and a trigger force of 0.1N. The instrumental data were collected using Texture Exponent 32
version 1.0.0.92 and the macro options texture exponent (Stable MicroSystems, Godalming, Surrey, UK) was employed to determine the MORSE values from the force-distance curves.

**Statistical analysis**

The association of the different parameters with respect to the occurrence of the NORM, MOD and SEV degrees of white striping was analyzed using Multinomial logistic regression model:

\[ z_i = \alpha_i + \beta_{i1}x_1 + \beta_{i2}x_2 + \ldots + \beta_{ik}x_k \]

where \( z_i \) is the log odds for the \( i^{th} \) dependent category with respect to the reference category; \( \alpha_i \) is the constant; \( 1, \ldots, k \) denote the independent variables, some of which may be interaction terms and \( \beta_{ik} \) is the logistic coefficient for the \( i^{th} \) category and \( k^{th} \) independent variable. The MOD and SEV degrees of white striping were considered as the dependent variables with NORM as the reference category. The independent variables considered in the model include the indicator variables representing the distinct levels of factors, the mean centered covariates and their two and three way interactions. The factors or the categorical variables used in the study were strain, feed, gender and chill hour. The covariates or continuous variables include the carcass/meat quality parameters such as RTC weight, length, width, \( H_1, H_2, pH_{24}, L_24^*, a_24^*, b_24^*, \) cook loss and MORSE. The continuous variables were centered to their respective means in order to avoid multicollinearity in the model. The data was analyzed using multinomial stepwise logistic regression procedure with forward selection option in PASW Statistics 18 (SPSS Inc., Chicago, IL, USA) which involves the maximum likelihood estimation. The result from the analysis is reported mainly as the odds ratio, 95% confidence interval (CI) and the respective \( P \) values. Odds is the ratio of the probability of an event of interest to the probability that the event will not
occur and odds ratios are the ratios of two odds comparing two groups. The odds ratio indicates the increased or decreased chance of a dependent category as a result of an increase in the continuous variable by one unit or with a categorical variable in comparison to a reference. An odds ratio >1 indicates an increased chance while <1 denotes a decreased chance. When the odds ratio is equal to 1, there is an equal chance for the category in question and the reference category. The estimated probability of occurrence of the three degrees of white striping was determined for all the categorical variables. For continuous variables showing significant effects, the data was grouped into bins and the average estimated probabilities were plotted to respective bins in order to visualize the pattern of relationship. Equal sized bin width was obtained using the interactive binning procedure in JMP statistical software version 9.0 (SAS Institute Inc.), setting the cut off at 10 percentile. The present study focuses on the association between the various factors (feeding regimen, strain, gender and chill hour) and meat quality attributes (RTC weight, length, width, H1, H2, pH24, L*24, a*24, b*24, cook loss and MORSE) with three different degrees of white striping. The statistical analysis and detailed description on the effect of these factors (feeding regimen, strain, gender and chill hour) on various carcass/meat quality attributes is presented in Brewer et al. (2012).

D. RESULTS AND DISCUSSION

**Relationship of white striping with various carcass and meat quality attributes**

The final multinomial logistic model obtained in the present study showed that the main carcass and meat quality factors (Table 2) that are significantly \( (P < 0.05) \) associated with MOD and SEV white striping are cranial thickness (H1) of the fillet and RTC weight while color b*24 value (which indicates the yellowness of the meat) was related \( (P < 0.05) \) to the occurrence of
SEV degree. Meanwhile, the model did not show any significant \((P > 0.05)\) association between length, width, caudal thickness \((H_2)\) of the fillet, \(\text{pH}\), color \(L^*_{24}\) and \(a^*_{24}\) values, cook loss and MORSE values with respect to the occurrence of three degrees of white striping. Cranial thickness of the fillet had a significantly \((P < 0.05)\) higher (highest OR values) influence on the occurrence of MOD (OR \(8.736; 95\% \text{ CI } 3.323\) to \(22.968\)) and SEV fillets (OR \(39.246; 95\% \text{ CI } 8.133\) to \(189.379\)) with reference to the NORM fillets (Table 2). This indicates that as the cranial thickness of the fillets increases, there is a greater probability that it could have a MOD or SEV degree of white striping (Figure 1). According to Lubritz (1997), fillet thickness had a much greater impact on fillet weight when compared to the length and width of fillet. Furthermore, Brewer et al., (2012) observed a higher correlation \((r = 0.84)\) between fillet weights and the cranial thickness of the fillets. These suggest that higher degrees of white striping (MOD and SEV) could be mainly associated with heavier or thicker fillets. This was in accordance with the findings from the previous studies (Bauermeister et al., 2009; Kuttappan et al., 2012b).

Kuttappan et al. (2012b) evaluated the effect of growth rate on the incidence of white striping and found that high-fat diet could increase the growth rate and fillet weight in birds resulting in decreased percentage of NORM and increased percentage of SEV fillets when compared to birds fed with low-fat diet. The results from the present study showed a similar trend in case of RTC weight (Figure 2). With reference to the NORM category, there was a significantly \((P < 0.05)\) higher chance of occurrence of MOD (OR \(1.004; 95\% \text{ CI } 1.001\) to \(1.006\)) and SEV (OR \(1.004; 95\% \text{ CI } 1.001\) to \(1.008\)) fillets as the RTC weight of the carcass increased. Even though, the OR (increase in chance for unit increase in the variable) is only slightly higher than 1, the range of the RTC (minimum RTC – \(1127\) g; maximum RTC – \(3708\) g) in the study is relatively large. So the net effect of RTC on the condition could be high which resulted in a higher \(P\) - value (0.004).
Kuttappan et al. (2011) observed that the higher degrees of white striping could be associated with increased ($P < 0.05$) occurrence of muscle damage characterized by myopathic changes in broiler breast fillets. The results from the present study imply that enhanced growth, resulting in greater RTC weight could have put more stress on the broilers resulting in muscle damage. Wilson, et al. (1990) suggested that the muscle from turkeys with increased growth may have outgrown their supporting systems which results in muscle damage. A reduction in the number of capillaries surrounding a single muscle fiber in the necrotic regions of turkey pectoralis major muscle was observed by Sosnicki and Wilson (1991). The study on the fiber area and capillary supply in broiler breast muscle in relation to productivity and ascites found that capillary density decreased in proportion to increase in fiber size and the chickens with higher percentage of breast muscle had a lower capillary density (Hoving-Bolink et al., 2000). This may result in a reduced supply of nutrients, oxygen and also slower removal of lactic acid from the muscles leading to muscle damages (Hoving-Bolink et al., 2000). However, further studies are required to confirm the details of tissue changes taking place in fast growing broiler breast fillets which result in higher degrees of white striping.

In addition, there was significantly ($P < 0.05$) greater chance that higher $b^{*}_{24}$ values are seen associated with SEV fillets when compared to the NORMs (Figure 3). The higher percentage of fat even on the dorsal or bone side of fillets with higher degrees of white striping (Kuttappan et al., 2011) could have contributed to this increased yellowness or $b^{*}_{24}$ values. However, further research is needed to determine the relationship between white striping and $b^{*}_{24}$ value. The occurrence of different degrees of white striping was not associated ($P > 0.05$) with changes in pH, color $L^{*}_{24}$ and $a^{*}_{24}$ values, cook loss and tenderness, which is in agreement with the findings of Bauermeister et al. (2009). White striping may not be causing any major
metabolic changes in muscle tissue which could have reflected in the case of pH and water holding capacity. However, the increased fibrosis in white striping fillets observed by Kuttappan et al. (2011) may give an impression of associated toughness which was not observed in the present study. Meanwhile, Kuttappan et al. (2011) also observed the degeneration of muscle fibers and an increase in fat, which together may have masked the effect of increased connective tissue. Another possibility is that since all the birds were grown to the same age, the increase in the connective tissue may not be to that extent to have a significant impact on tenderness of the meat used in the study.

**Incidence of white striping and the associated factors**

Among the total birds used in this experiment, 55.8% showed some degree of white striping with MOD and SEV categories as 47.5 and 8.3%, respectively. The stepwise procedure used for logistic regression modeling showed that strain differences were significantly \( P < 0.05 \) associated with the incidence of white striping. The details of the parameters (RTC weight, \( H_1 \) and \( b^{*24} \) value) which were significantly \( P < 0.05 \) affecting the occurrence of three degrees of white striping with respect to various factors like strain, gender, feeding regimen and chill hour are presented in Table 3. This could help to derive meaningful conclusions from the final logistic regression model. However, the mean comparison for these variables was not performed with respect to three degrees of white striping because of the large disparity in number of samples (n) in each group (Table 4). Among the four different strains, Strain A showed the highest percentage of NORM fillets while Strain B showed the lowest percentage of NORM but the highest percentage of SEV fillets (Table 1). During the stepwise modeling, Strain A was considered as the reference because it had the highest percentage of NORM fillets. Between the strains, there were differences in the percentage of occurrence of the three degrees of white
striping (Table 1) which could be an impact of the differences in RTC weight and cranial fillet thickness (Table 3). However, when the OR of occurrence of MOD condition with reference to NORM was considered, Strain B had a significantly \((P = 0.013)\) higher chance (OR 3.595; 95% CI 1.306 to 9.894) than Strain A which resulted in the inclusion of the factor into the model (Table 2). Furthermore, there was a significant interaction between the strain and RTC weight. The probability of occurrence of NORM, MOD and SEV degrees with respect to strain and RTC weight reveal that strain B and C had a different pattern in the incidence of white striping with respect to A (Figure 4). In the case of Strain C, the probability of occurrence of NORM, MOD and SEV degrees were almost consistent regardless of the increase in RTC weight. Surprisingly, such an interaction was not observed between strain and cranial thickness of the fillet which suggests that an increase in fillet thickness resulted in increased chance of higher degrees of white striping, regardless of the strain. Since Strain C was bred to be moderate yielding classic type broiler, while the other strains were high yielding broilers, differences in the growth pattern in Strain C may have resulted in such a significant effect in case of MOD \((P = 0.001)\) and SEV degrees \((P = 0.001)\) (Table 2). On the other hand, Strain B had the highest RTC weight and cranial fillet thickness among the strains studied (Table 3). The comparatively heavier fillets in these birds may have resulted in the higher occurrence of MOD fillets even at lower RTC weights (Figure 4) which could have resulted in a significant \((P = 0.023)\) strain to RTC interaction (Table 2). Also, the probability of the occurrence of SEV degree spiked up to 80% at the highest RTC weight and on an average, there was more than 60% chance that a fillet obtained from strain B bird will have either MOD or SEV degrees of white striping (Figure 4). Some of the earlier studies reported that the occurrence of hereditary muscular dystrophy in chicken could result in the occurrence of white striations (Asmundson and Julian, 1956; Julian and Asmundson,
1963) which are similar to the gross lesions of white striping. However, the occurrence of hereditary muscular dystrophy is confined to strains which are homozygous for autosomal recessive gene (am) even though the phenotypic expression could depend on other modifying genes (Asmundson and Julian, 1956; Wilson et al., 1988). Also, there are few reports on the occurrence of hereditary muscular dystrophy in modern commercial broiler strains. Meanwhile, the results from the present study showed that there is no strain predilection for the incidence of white striping. Nonetheless, the difference in the percentage of incidence between strains could be related to the difference in growth pattern. So, the white striping seen in modern broiler may have a different etiology when compared to hereditary muscular dystrophy.

The final logistic regression model (Table 2) also showed that the effect of gender, feeding regimen and chill hour on the occurrence of white striping were not significant ($P > 0.05$). In comparison to females, male birds showed the lowest percentage of NORM fillets and the highest percentage of SEV fillets (Table 1). However, the gender was not considered as a significant factor during the stepwise modeling procedure (Table 2). This implies that the difference in the percentages could be a manifestation of the higher RTC weight and thicker fillets in males when compared to females (Table 3). The differences in the occurrence of three degrees of white striping were not that apparent with respect to the chill hour and diet treatments (Table 1) and these two parameters were not significant in the logistic regression model, as well. In the present study, the diet treatments had only a lesser impact on the uniformity of RTC weight and the cranial fillet thickness (Brewer et al., 2012), as indicated by the standard error (Table 3). This may be the reason why the occurrence of three degrees of white striping did not seem to be affected by the feeding regimen as a result it was not significant and therefore not included in the final model. A similar trend can be seen in case of chill hour as well. White
striping could be a condition existing prior to processing so that it was expected not to have an effect on the incidence. In addition, the occurrence of white striations is seen in case of the dietary deficiency of vitamin E and the associated nutrients referred to as nutritional myopathy (Dam et al., 1952; Machlin and Shalkop, 1956; Klasing, 2008). However, the present study used diet formulations which contained vitamin E levels equal to or exceeded the National Research Council (1994) recommendations (Brewer et al., 2012). Furthermore, the occurrence of higher degrees for white striping was observed regardless of the diet treatments. So, the chance of vitamin E deficiency related to the occurrence of white striping is less. Further studies are needed to confirm the impact of different dietary vitamin E level on the occurrence of white striping.

E. CONCLUSION

The results from the present study showed that the risk of occurrence of MOD and SEV degrees are associated with heavier birds with thicker fillets. However, there are differences with respect to strains which could be influenced by the growth pattern. The occurrence of SEV white striping may also be accompanied with increase in yellow color of the fillets at 24 h PM. Industrial/PF regimens as well as chill hour during processing do not influence the incidence of the condition. In addition, results of this study suggest that the fillets with three degrees of white striping are not associated with any significant difference in meat quality attributes like pH\textsubscript{24}, L\textsuperscript{*}\textsubscript{24}, a\textsuperscript{*}\textsubscript{24}, cook loss and tenderness.
F. REFERENCES


Table 1: Frequency and the probability of occurrence of three\(^1\) degrees of white striping (Kuttappan et al., 2012a) with respect to the factors or categorical independent variables

<table>
<thead>
<tr>
<th>Factors</th>
<th>Frequency*</th>
<th></th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NORM</td>
<td>MOD</td>
<td>SEV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>112 (59.3)</td>
<td>72 (38.1)</td>
<td>5 (2.6)</td>
<td>189</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>50 (27.2)</td>
<td>99 (53.8)</td>
<td>35 (19.0)</td>
<td>184</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>85 (49.1)</td>
<td>83 (48.0)</td>
<td>5 (2.9)</td>
<td>173</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>80 (41.5)</td>
<td>97 (50.2)</td>
<td>16 (8.3)</td>
<td>193</td>
<td></td>
</tr>
<tr>
<td>Feeding regimen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Industrial-type feeding</td>
<td>174 (45.3)</td>
<td>180 (46.9)</td>
<td>30 (7.8)</td>
<td>384</td>
<td></td>
</tr>
<tr>
<td>Phase feeding</td>
<td>153 (43.1)</td>
<td>171 (48.2)</td>
<td>31 (8.7)</td>
<td>355</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>199 (53.2)</td>
<td>157 (42.0)</td>
<td>18 (4.8)</td>
<td>374</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>128 (35.1)</td>
<td>194 (53.1)</td>
<td>43 (11.8)</td>
<td>365</td>
<td></td>
</tr>
<tr>
<td>Chill hour</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4h</td>
<td>157 (42.7)</td>
<td>180 (48.9)</td>
<td>31 (8.4)</td>
<td>368</td>
<td></td>
</tr>
<tr>
<td>6h</td>
<td>170 (45.8)</td>
<td>171 (46.1)</td>
<td>30 (8.1)</td>
<td>371</td>
<td></td>
</tr>
</tbody>
</table>

\*N (% based on the total number in the respective rows)

\(^1\)NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Table 2: Odd ratio (OR), 95% confidence interval (CI) and the probability ($P$ value) level for variables in the model with respect to the three degrees of white striping\(^1\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>MOD(^a)</th>
<th></th>
<th></th>
<th>SEV(^a)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>$P$ value</td>
<td>OR</td>
<td>95% CI</td>
<td>$P$ value</td>
</tr>
<tr>
<td>$H_1$ (cm)</td>
<td>8.736</td>
<td>3.323-22.968</td>
<td>&lt;0.001</td>
<td>39.246</td>
<td>8.133-189.379</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RTC (g)</td>
<td>1.004</td>
<td>1.001-1.006</td>
<td>0.004</td>
<td>1.004</td>
<td>1.001-1.008</td>
<td>0.019</td>
</tr>
<tr>
<td>$b^*_{24}$ value</td>
<td>1.087</td>
<td>0.873-1.354</td>
<td>0.456</td>
<td>1.856</td>
<td>1.213-2.841</td>
<td>0.004</td>
</tr>
<tr>
<td>[Strain A](^b)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>[Strain B]</td>
<td>3.595</td>
<td>1.306-9.894</td>
<td>0.013</td>
<td>3.343</td>
<td>0.451-24.772</td>
<td>0.238</td>
</tr>
<tr>
<td>[Strain C]</td>
<td>2.261</td>
<td>0.938-5.451</td>
<td>0.069</td>
<td>0.206</td>
<td>0.005-8.122</td>
<td>0.399</td>
</tr>
<tr>
<td>[Strain D]</td>
<td>1</td>
<td>0.417-2.397</td>
<td>1</td>
<td>1.874</td>
<td>0.328-10.709</td>
<td>0.48</td>
</tr>
<tr>
<td>[Strain A] * RTC(^b)</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
</tr>
<tr>
<td>[Strain B] * RTC</td>
<td>0.996</td>
<td>0.992-0.999</td>
<td>0.023</td>
<td>0.999</td>
<td>0.994-1.004</td>
<td>0.638</td>
</tr>
<tr>
<td>[Strain C] * RTC</td>
<td>0.995</td>
<td>0.992-0.998</td>
<td>0.001</td>
<td>0.988</td>
<td>0.982-0.995</td>
<td>0.001</td>
</tr>
<tr>
<td>[Strain D] * RTC</td>
<td>0.998</td>
<td>0.995-1.001</td>
<td>0.218</td>
<td>0.997</td>
<td>0.992-1.001</td>
<td>0.168</td>
</tr>
</tbody>
</table>

\(^1\)NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree

\(^a\)The reference category is NORM

\(^b\)This parameter is set to zero because it is redundant (used as reference)
Table 3: Summary of the meat quality attributes significantly \((P < 0.05)\) associated with the occurrence of white striping with respect to breed, feed treatment, gender and chill hours

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Strain</th>
<th>Feed</th>
<th>Gender</th>
<th>Chill hour</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>RTC wt.(g)</td>
<td>Mean</td>
<td>2379.3</td>
<td>2543.04</td>
<td>2364.89</td>
</tr>
<tr>
<td></td>
<td>SE(^1)</td>
<td>28.02</td>
<td>27.09</td>
<td>27.65</td>
</tr>
<tr>
<td>H(_1) (cm)</td>
<td>Mean</td>
<td>2.76</td>
<td>3.07</td>
<td>2.71</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>b(_{24}) value</td>
<td>Mean</td>
<td>2.4</td>
<td>2.76</td>
<td>2.37</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.11</td>
<td>0.12</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^1\) Standard error
Table 4: Mean and standard error (SE) of meat quality attributes of fillets classified as NORM, MOD and SEV degrees\(^1\) of white striping

<table>
<thead>
<tr>
<th>Attributes</th>
<th>Degree of white striping</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NORM (n=327)</td>
<td>MOD (n=351)</td>
<td>SEV (n=61)</td>
</tr>
<tr>
<td>RTC wt. (g)</td>
<td>Mean</td>
<td>2293.67</td>
<td>2516.6</td>
<td>2766.98</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>19.03</td>
<td>18.88</td>
<td>48.54</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>Mean</td>
<td>19.03</td>
<td>19.16</td>
<td>19.23</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.06</td>
<td>0.06</td>
<td>0.13</td>
</tr>
<tr>
<td>Width (cm)</td>
<td>Mean</td>
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<td>9.84</td>
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</tr>
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</tr>
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<td>H(_1) (cm)</td>
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<tr>
<td>L(_{24}) value</td>
<td>Mean</td>
<td>52.63</td>
<td>52.76</td>
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<tr>
<td></td>
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<td>0.15</td>
<td>0.16</td>
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<tr>
<td>a(_{24}) value</td>
<td>Mean</td>
<td>3.75</td>
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<td>SE</td>
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<td>0.05</td>
<td>0.1</td>
</tr>
<tr>
<td>b(_{24}) value</td>
<td>Mean</td>
<td>2.37</td>
<td>2.72</td>
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<td>Mean</td>
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\(^1\)NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Figure 1: Probability of occurrence of NORM ( ), MOD ( ) and SEV ( ) fillets with respect to cranial thickness of fillet
Figure 2: Probability of occurrence of NORM (●), MOD (□) and SEV (●) fillets with respect to RTC weight
Figure 3: Probability of occurrence of NORM ( ), MOD ( ) and SEV ( ) fillets with respect to $b^*_{24}$ values
Figure 4: Probability for NORM (●), MOD (□) and SEV (★) fillets with respect to RTC weight in different strains.
V. INFLUENCE OF GROWTH RATE ON THE OCCURRENCE OF WHITE STRIPING IN BROILER BREAST FILLETS

A. ABSTRACT

White striping refers to the occurrence of different degrees of white striations on broiler breast fillets and thighs of larger broilers, yet little is known about its causes. Thus, the objective of the study was to estimate the occurrence of normal (NORM), moderate (MOD), and severe (SEV) degrees of white striping with respect to the growth rate of broilers and to compare their proximate composition without the confounding effect of diet. Straight-run, day-old chicks (n = 280) were randomly assigned to either a low- (LED) or high-energy (HED) diet (5 replicates of 28 birds/dietary treatment). Birds were processed at 54 d of age, and live weight, deboned fillet weight, and occurrence of white striping were recorded. As expected, birds fed the HED had lower ($P < 0.05$) feed conversion ratio than birds fed LED (2.08 vs. 2.28). Also, HED-fed birds had heavier ($P < 0.05$) live and fillet weights when compared to the LED-fed birds. A greater ($P < 0.05$) percentage of breast fillets from LED-fed birds were scored NORM, whereas HED-fed birds produced a greater ($P < 0.05$) percentage of SEV fillets. Fillet weight and yield (percent of live weight) increased ($P < 0.05$) as the degree of white striping increased from NORM to SEV. Additionally, NORM fillets had greater ($P < 0.05$) lipid, and lower ($P < 0.05$) protein, content when compared to SEV fillets. Also, NORM fillets had greater ($P < 0.05$) percentages of SFA than SEV fillets; however, proportions of all MUFA, as well as linoleic and linolenic acids, were greater ($P < 0.05$) in SEV than NORM fillets. These results suggest that an increased growth rate results in increased occurrence of higher degrees of white striping in broiler breast fillets, and the various degrees of white striping are associated with differences in chemical composition of breast fillets.
B. INTRODUCTION

The per capita availability of chicken in the U.S. more than doubled between 1970 and 2005, while that of the red meats declined (Wells and Buzby, 2008). One of the major reasons for this change was that poultry meat was a healthier alternative to red meats. In fact, a joint report from Royal College of Physicians of London and the British Cardiac Society (RCP-BCS, 1976) recommended replacing red meat with more poultry to reduce the incidence of coronary heart diseases. Furthermore, the fat deposited in poultry is mainly associated with skin which makes it easier to be removed; thereby, substantially reducing fat consumption (Sams and Alvarado, 2010). Besides the perceived health benefit, ease of cooking and availability at reasonable prices also contributed to poultry meat becoming more popular among consumers (Haley, 2001). Increased consumer demand has forced producers to explore methods for increasing their production output. Obviously, increasing production efficiently is important to growers, and a number of studies have shown that increasing the energy level (fat content) in poultry diets could improve growth rate and feed efficiency in birds (Mabray and Waldroup, 1981; Deaton et al., 1983; Deaton and Lott, 1985; Holsheimer and Veerkamp, 1992; Summers et al., 1992; Holsheimer and Ruesink, 1993). However, challenging birds to attain high body weight (BW) within a short period of time could cause various meat quality problems.

Recent publications reported concerns that modern poultry meat contains more fat compared to that produced several years ago (Ungoed-Thomas, 2005; Wang et al., 2010). According to Wang et al. (2010), the modern chicken carcass has almost 2- to 3-times more energy contributed by fat when compared to the energy from protein. They suggested that the selection for fast weight gain, high-energy diets, and *ad libitum* access to feed without adequate exercise could have resulted in the increase in fat content. Crawford et al., (2010) pointed out
that the chicken thigh eaten in 2006 might contain 100 more calories compared to the one during 1970s, and the management practices would have resulted in weight gain as fat infiltration with associated muscle loss. Recently, fillets from broiler birds of market age were identified with varying degrees of white striations, seen parallel to muscle fibers, mainly on the chicken fillets and thighs. The condition is referred to as white striping, and based on the visual scoring of the severity of condition, fillets can be classified as normal or no striping (NORM), moderate (MOD), or severe (SEV) fillets (Kuttappan et al., 2012). Interestingly, the reduced visual acceptance of the MOD and SEV degrees of white striping could be a potential reason for the consumer dislike and reduced purchase intent because of the fatty, or marbled, appearance of the product (Kuttappan et al., 2012). The condition is mainly associated with heavier birds and high incidences (> 50%) can occur, especially in big birds (Kuttappan et al., 2009). Bauermeister et al. (2009) reported a higher incidence in birds processed at 8 than 6 wk of age, which could be a manifestation of the greater BW at older ages. Processing big birds for the heavy debone market is more common these days, with meat being used for portioning and further processing. So, the occurrence of white striping in broiler breast fillets could adversely affect the poultry meat market and result in great economic loss. Therefore, it is important to determine the impact of increased growth rate on the incidence of the white striping condition. Furthermore, there could be associated compositional differences in meat associated with white striping. Thus, the objectives of this study were to estimate the occurrence of NORM, MOD, and SEV degrees of white striping with respect to the growth rate of broilers, and to compare the proximate and fatty acid composition of breast fillets with the three degrees of white striping occurrence.
C. MATERIALS AND METHODS

Experimental design

All procedures were approved by the University of Arkansas Institutional Animal Care and Use Committee. For this experiment, 280 straight-run, day-old chicks of a commercial broiler strain (Cobb 500) were assigned to either low- (LED) or high-energy (HED) dietary treatment. Two series of diets were formulated. The first diet was formulated based on the suggested needs for male broilers with low performance and the second for male broilers with high performance (Rostagno et al. 2005). The low performance diets were assigned a nutrient density commensurate with approximately 0.5% supplemental poultry oil while the second group a nutrient density commensurate with approximately 6% supplemental poultry oil. All diets were fortified with complete vitamin and trace mineral mixes obtained from commercial sources. Composition and calculated nutrient content of the diets is shown in Table 1. The different calorie contents in the two diet treatments were meant to produce difference in the growth rate of these birds. There were 5 replicates of each dietary treatment (28 birds/treatment). Weights of birds and feed intake were recorded after starter, grower, and finisher periods in order to evaluate growth rate and feed conversion (feed: gain). All birds had ad libitum access to feed and water.

At 54 d of age, all birds were processed using commercially accepted methods at the University of Arkansas Poultry Processing Pilot Plant (Mehaffey et al., 2006). Meat samples were collected immediately after scalding from the ventral (skin side) part of the cranial region of right fillets (pectoralis major) for conducting proximate analysis. All the carcasses were pre-chilled (12°C for 15 min), chilled (1°C for 75 min), and subsequently deboned at 2h postmortem. Live body and left-side fillet weights were recorded, and occurrence of white striping in each
fillet was scored as normal (NORM), moderate (MOD), or severe (SEV) based on the criteria described by Kuttappan et al. (2012). The fillets that did not show any distinct white striations were scored as NORM, whereas those with small thin lines were considered as MOD; SEV fillets had thick white striations covering most of the surface area.

**Proximate analysis**

Most representative samples from each degree (NORM, MOD, and SEV) of white striping were selected from only the HED-fed birds for proximate and fatty acid analyses. The intention was to avoid the confounding effect, if any, due to the two diet treatments. Proximate composition of the raw breast fillets was determined at the University of Arkansas Central Analytical Laboratory. Breast fillet samples (approximately 40 g) were weighed in plastic containers and placed in a freeze-dryer (Virtis Genesis, Gardiner, NY) set at -10°C, and samples were allowed to freeze-dry for 8 d until the pressure reached 0 mm of Hg. Moisture percentage was determined from the difference between sample weights before and after freeze-drying, and was expressed as percentage of the initial fresh weight. Fat, protein, and ash contents were estimated using ether extraction (AOAC #920.39C), combustion (AOAC #990.03), and incineration (AOAC #923.03) methods, respectively (AOAC, 1990), and were reported as percentages on a dry matter (DM) basis.

**Fatty acid analysis**

Freeze-dried samples were subsequently pulverized and used for the estimation of fatty acid profile using the procedure explained by Apple et al. (2009), with some modifications. Duplicate 225-mg samples were placed into 16 × 125-mm, screw capped tubes and mixed with 2mL of 0.2 M potassium hydroxide in anhydrous methanol. Before that, 1mg of
glyceryltritridecanoate (Supelco T-3882) was added to each tube as internal standard. Fatty acids were transesterified to methyl ester by incubating at 50°C for 45 min, with intermittent vortexing (2 to 3 times /min). After cooling to room temperature, fatty acid methyl esters (FAME) were extracted by adding 1 mL of saturated sodium chloride, followed by 1 mL of hexane. Tubes were then centrifuged at 1,100 × g (20°C) for 5 min to separate the phases. A portion of the hexane layer containing FAME was transferred to a gas chromatograph (GC) vial containing a 1.0-mm bed of anhydrous sodium sulfate. A GC (Model HP 5890 Series II GC, with an HP-7673 automatic injector and HP-3365 software; Hewlett-Packard, Avondale, PA), fitted with FID detector and 100-m capillary column (0.25mm i.d.; Model SP-2560 fused-silica capillary column, Supelco Inc., Bellefonte, PA) and using HE (20cm/s) as the carrier gas (1:50 split ratio), was used to separate the individual FAME. Initially, the oven temperature was set at 150°C for 5 min. Then, the temperature was increased to 194°C at 4°C/min for 15 min and subsequently to 235°C at 2.5°C/min for 16.25 min. Purified standards used to qualify the peaks were obtained from Nu-Check Prep (Elysian, MN) and Matreya (Pleasant Gap, PA), as well as a FAME mixture (Supelco 37 Component mix, Cat no. 4-7885). Concentrations of FAME in samples were determined using internal standard methodology. Total fatty acid and individual FAME concentrations were reported as mg of FAME/g of dry sample and FAME weight percentages, respectively. The Δ⁹ desaturase enzyme activity for 16-carbon \([([16:1]/[16:0+16:1])× 100] \) and 18-carbon fatty acids \([(18:1c9)/(18:0 + 18:1c9))]× 100] \) as well as the elongase enzyme activity in the chain lengthening of 16- to 18-carbon fatty acids \([(18:0 + 18:1c9)/(16:0 + 16:1 + 18:0 + 18:1c9)]× 100] \) were estimated (Malau-Aduli et al., 1998). The total percentages of saturated (SFA = 14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0 + 21:0), monounsaturated (MUFA = 14:1 + 16:1c + 16:1t + 18:1c11 + 18:1c9 + 18:1t9 + 20:1c11) and
polyunsaturated (18:2n-6 + 18:2c9c11 + 18:2c9t11 + 18:3n-3 + 18:3n-6 + 20:2n-6 + 20:3n-3 + 
20:3n-6 + 20:4n-6 + 20:5n-3 + 22:5n-3 + 22:6n-3) fatty acids were calculated. Furthermore, the 
ratio between the major PUFA and SFA [P/S = ([18:2n-6] + [18:3n-3]) / ([12:0] + [14:0] + [16:0] 
+ [18:0])] (Enser et al., 2000), \( \sum_{n-6}/\sum_{n-3} \) [(18:2n-6 + 18:3n-6 + 20:2n-6 + 20:3n-6 + 20:4n-
6)/(18:3n-3 + 20:3n-3 + 20:5n-3 + 22:5n-3 + 22:6n-3)] and n-6/n-3 (18:2n-6/18:3n-3) ratios were 
determined to compare the nutritive value of the breast fillets.

**Statistical analysis**

A completely randomized design was used and analysis of the data was performed with 
GLM. Least square means were separated using either Student’s t test or Tukey’s HSD test 
depending on the number of treatments used (JMP version 9.0, SAS Institute Inc.). Chi-square 
analysis was conducted to assess the occurrence of white striping in the two diet treatments. 
Because birds from only the HED were used to compare the proximate composition and fatty acid 
profile of the meat, degree of white striping served as the only main effect. Significance was 
determined at \( P < 0.05 \) for all parameters. The pens were considered as replicates for determining 
the effect of diet treatments on body weight gain and feed conversion ratio during the entire 
period of the study. Meanwhile, individual birds were used as replicates for estimating the effect 
of various treatments on carcass quality and proximate composition.

**D. RESULTS AND DISCUSSION**

**Growth performance and occurrence of white striping**

Birds fed HED had greater \( (P < 0.05) \) BW than LED-fed birds at 18, 32, and 54 d of age 
(Figure 1). In addition, the HED-fed had lower \( (P < 0.05) \) feed conversion ratios than the LED-
fed birds during the starter and finisher periods (Figure 2). For the overall growth period (0 to 54
d), LED-fed birds had greater \((P < 0.05)\) feed conversion ratio (2.28 vs. 2.08) and a lower BW gain (2.72 vs. 2.98 kg/bird) than HED-fed birds. The average daily gain (ADG) was greater \((P < 0.05)\) in HED-fed than LED-fed birds (0.055 vs. 0.050 kg/d). Moreover, HED-fed birds were heavier \((P < 0.05)\) at slaughter, and produced heavier \((P < 0.05)\) fillets than LED-fed birds though fillet yield was not impacted in this study (Table 2). These results clearly indicate that the HED-fed birds grew more rapidly than their LED-fed counterparts. In fact, a number of studies have repeatedly demonstrated that feeding birds a high-energy diet results in improvements in feed conversion efficiency, growth rate, and breast meat yield when compared to birds fed reduced energy diets (Mabray and Waldroup, 1981; Deaton et al., 1983; Holsheimer and Veerkamp, 1992; Summers et al., 1992; Holsheimer and Ruesink, 1993).

After processing and deboning, fillets were categorized as NORM, MOD, and SEV based on the degree of white striping. The HED-fed birds produced a greater \((P < 0.05)\) percentage of SEV fillets and a lower \((P < 0.05)\) percentage of NORM fillets compared to LED-fed birds; however, the percentage of MOD fillets did not differ between birds fed HED and LED (Table 2). It is important to note that the MOD fillets represented the largest proportion of fillets in HED-fed (65.9%) and LED-fed birds (51.1%). Moreover, fillets weights and yields increased \((P < 0.05)\) as severity of striping increased in breast fillets (Table 3). Kuttappan et al. (2009) reported similar results, suggesting that the higher degrees of white striping were mainly associated with thicker or heavier fillets. These results suggest that the heavier fillet weights associated with increased growth rates in market broilers is also associated with the increased incidence of higher degrees of white striping. Furthermore, the histological studies conducted by Kuttappan et al. (2011) suggested that a higher degree of white striping is associated with muscle damage and myopathic changes. This implies that the enhanced growth rate in birds could result
in muscle damage which is manifested grossly as white striping. The intense selection for rapid
growth rate and meat yield in birds could be accompanied by the lack of adequate development
in the capillary or other supporting system resulting in growth induced myopathy (Mahon, 1999).
Increased growth rate and the associated ischemia resulting muscle damage are reported in
turkeys (Sosnicki et al., 1989; Sosnicki et al., 1991). However, the above studies detected the
muscle damage only in microscopic or ultra-structural levels while the gross appearance of those
muscles was normal.

**Proximate composition of fillets with different degrees of white striping**

There was an increase ($P < 0.05$) in the fat content, with a corresponding decrease ($P <
0.05$) in the protein content, as the degree of striping increased from NORM to SEV (Table 3).
Consequently, the fat: protein ratio also increased ($P < 0.05$) as the degree of white striping
occurrence increased from NORM to SEV; however, moisture and ash contents did not differ
among striping occurrence scores. The difference in the proximate composition could be a sequel
to the myopathic changes seen in higher degrees of white striping. The degeneration of the
muscle fibers could have resulted in the reduced protein content although the etiology of increase
in fat content is not clear. The reduction in protein content (may be an indication of muscle fiber
degeneration or atrophy) could have resulted in creating more space for the adipocytes to
expand, thereby allowing for increased fat deposition. However, it is possible that there may be
systemic changes associated with this increased fat deposition. The contribution of adipocytes in
chicken to *de novo* lipogenesis is minute and is typically not altered much by any dietary
changes (Leveille et al., 1975; Saadoun and Leclercq, 1987; Griffin et al., 1992). Therefore, the
increased deposition of fat in chicken adipocytes could be due to an increase in the amount of
plasma lipid substrate reaching the adipocytes or an increased break down of the plasma lipid at
the adipocytes leading to increased uptake and storage in the adipose tissue (Hermier, 1997). Furthermore, the source of circulating plasma lipids could be either the lipogenesis in the liver or of the dietary origin. On the other hand, the increased catabolism of lipoprotein particles at the adipocyte is due to an increase in the action of lipoprotein lipase (LPL). Hermier et al. (1989) reported that the hyperplasia of adipocytes in fat birds resulted in enhanced LPL activity which subsequently led to increased fatty acid uptake in adipose tissue. In case of white striping, the degeneration of muscle fibers during myopathy (Kuttappan et al., 2011) could be associated with differentiation of muscle stem cells to adipocytes (Asakura et al., 2001; Shefer et al., 2004; Hosoyama et al., 2009) resulting in adipocyte hyperplasia. Because the present study used fillet from only HED-fed birds for proximate analysis, the increased fat deposition observed in SEV in comparison to the NORM fillets could either be due to an increased lipogenesis in liver or an increased uptake of the circulating fat due to hyperplasia of adipocytes (may be due to a greater LPL activity) as a result of myopathic changes. However, further studies are needed to know more about the source of increase in fat deposition seen in fillets with higher degrees of white striping.

**Fatty acid profile of NORM and SEV fillets**

Fatty acid composition of NORM and SEV fillets were compared in order to determine whether there was any difference with respect to the nutritive value. The NORM fillets had greater \((P < 0.05)\) weight percentages of saturated fatty acid (SFA) but lower \((P < 0.05)\) percentages of monounsaturated fatty acids (MUFA) when compared to SEV fillets (Table 4). Among the individual SFA measured, the proportions of myristic, pentadecanoic, and arachidic acids were greatest \((P < 0.05)\) in SEV fillets, whereas palmitic and stearic acids were greatest \((P < 0.05)\) in NORM fillets. Palmitic and stearic acids are the major fatty acids in animal tissue that
are the result of *de novo* lipogenesis (Bruss, 1997). Similarly, the MUFA, oleic and elaidic acids were greater (*P* < 0.05) in SEV fillets, which contributed to greater percentage of total MUFA observed in SEV fillets. The estimation of desaturase enzyme activities indicated that SEV fillets had greater (*P* < 0.05) Δ⁹ desaturase (for 18-carbon fatty acids) and elongase activities than NORM fillets, which may have contributed to the greater proportions of oleic and elaidic acids.

On the other hand, a large proportion of the SFA and MUFA composition in the fat of monogastric animals originate from fats and oils in the animal diet (Wood and Enser, 1997; Nürnberg et al., 1998). Even though fillets came from birds of similar genotype fed the same diet, it is plausible there may be differences in the uptake of dietary fatty acids due to differences in adipocyte activity. However, results of this study cannot be used to distinguish whether the difference in SFA and MUFA observed in NORM and SEV fillets can be attributed to alterations in lipogenesis or dissimilarity in the uptake of dietary fat into the tissues.

It has been known that animals are not capable of synthesizing polyunsaturated fatty acids (PUFA), like linoleic and α-linolenic acids (Cook, 1996); so, the primary source of these PUFA is diet. Interestingly, the weight percentage of total PUFA did not differ between NORM and SEV fillets (Table 4). However, the percentages of linoleic acid and α-linolenic acids were greater (*P* < 0.05) in SEV than NORM fillets. Because the fillets for measuring fatty acid profiles were taken only from HED-fed birds, the observed differences in linoleic acid and α-linolenic acid percentages could presumably be due to the increased uptake of dietary fatty acids into adipocytes in the SEV fillets. Furthermore, according to Wood and Enser (1997), α-linolenic acid is the precursor of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA); however, SEV fillets had less (*P* < 0.05) amounts of EPA and DHA than NORM fillets, despite having the greatest proportion of α-linolenic acid. Rymer and Givens (2005) also failed to discern an
associated between increased concentrations of \( \alpha \)-linolenic acid in animal tissues and increased EPA and DHA concentrations in muscle tissue. Presumably, the lack of relationship may be due to the accumulation of synthesized EPA and DHA in liver and other tissues (Ajuyah et al., 1993). Furthermore, Hulbert et al. (2002) reported a negative correlation between DHA concentration in skeletal muscle and body size, and they attributed this inverse relationship to the need of greater amounts of DHA (for the functioning of sodium and calcium pumps) for maintaining a high metabolic rate in smaller birds. In the present study, HED-fed birds with heavier body weights produced a greater number of SEV fillets; therefore, the difference in size between birds producing NORM and SEV fillets may have contributed to the observed differences in the weight percentages of EPA and DHA, irrespective of the lower percentage of \( \alpha \)-linolenic acid in NORM samples. The linoleic and \( \alpha \)-linolenic acids, and their long-chain PUFA derivatives, are major components of the cell membranes, and act as precursors for various eicosanoids associated with inflammatory processes (Simopoulos, 2000, 2008). Perhaps, there might be some relationship between the muscle damages in the SEV birds Kuttappan et al. (2011) and its fatty acid profile. In fact, all these observations suggest that there are differences in fatty acid profile between NORM and SEV samples, but source of the variation remains unclear.

According to a recent report from the United States Departments of Agriculture and Health and Human Services (USDA-DHHS, 2010), the caloric intake of Americans has exceeded their daily energy requirements, which has contributed greatly to the obesity epidemic. Interestingly, the report ranks chicken and chicken-mixed dishes third among the top dietary sources of daily calories. In this light, the greater amount of calories discovered in fillets with the SEV degree of white striping (Table 3) can have an impact on the nutritional value of the poultry meat. Although the difference in caloric density between NORM and SEV fillets was
numerically small (387.38 vs. 400.94 kcal/100g on DM basis), this difference could be exasperated due to the confounding effect from various growth-promoting, high-nutrient diet formulations. It has been recommended to replace the saturated and \textit{trans}-polyunsaturated fat in human diets with unsaturated fatty acids (mainly PUFA) due to their health benefits (Hu et al., 2001; Reddy and Katan, 2004; Xu et al., 2006; Jakobsen et al., 2009; USDA-DHHS, 2010). Both linoleic and \(\alpha\)-linolenic acids are essential fatty acids in the human diet, and there appears to be competition between these two fatty acids for incorporation into tissue lipids (Cook, 1996).

Furthermore, increasing the level of \(\alpha\)-linoleic acid is beneficial because its derivatives, EPA and DHA, are involved in fetal neurodevelopment, and have been shown to reduce blood thrombosis and cardiovascular disease in human beings (Wood and Enser, 1997; Yokoyama et al., 2007; Gissi-Hf, 2008). So, a balance between levels of linoleic and \(\alpha\)-linolenic acids in diet is essential. According to Kouba et al. (2003), the London Department of Health and Social Security recommends that a healthy human diet should have a P/S ratio \(\geq 0.4\) and n-6/n-3 ratio between 1 and 4. In the present study, NORM fillets, with greater proportions of SFA, had elevated \((P < 0.05)\) UFA: SFA and reduced \((P < 0.05)\) P/S when compared to the SEV fillets (Table 5). In addition, NORM fillets had a greater linoleic: \(\alpha\)-linolenic ratio and a lower \((P < 0.05)\) \(\sum n\text{-}6:\sum n\text{-}3\) ratio (Table 5), in spite of the fact that NORM fillets had greater proportions of EPA and DHA. From a nutritional point of view, NORM fillets may have greater amounts of SFA, but NORM fillets are also higher in EPA and DHA. Dietary recommendations put forward by the USDA-DHHS (2010) suggest replacing part of meat and poultry in human diets with seafood mainly because of the nutrition benefits of the latter due to the higher amounts of EPA and DHA (Mozaffarian and Rimm, 2006). Therefore, the reduced level of EPA and DHA associated with the SEV fillets needs immediate attention. Further studies are needed in order to estimate the
source of variations and also the effect of various diet formulations on the fatty acid content of poultry meat with difference degrees of white striping.

In conclusion, the results of this experiment imply that feeding a HED can produce birds with greater BW and lower feed conversion ratios. The augmented growth rate induced by the high-calorie diet can increase the occurrence of white striping in broiler breast fillets. In addition, the higher degrees of white striping were associated with a greater amounts of lipid and lower amounts of protein, resulting in higher net calorie content in the meat. There were differences in the fatty acid composition of NORM and SEV degrees of white striping; however, additional research is warranted to elucidate the possible source(s) and nutritional significance of these changes.
E. REFERENCES


Table 1: Composition (% DM) of the low- (LED) and high-energy (HED) diets.

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<td>Soybean meal</td>
<td>32.360</td>
<td>39.199</td>
<td>30.012</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.274</td>
<td>0.175</td>
<td>0.246</td>
</tr>
<tr>
<td>Defluorinated phosphate</td>
<td>1.735</td>
<td>1.882</td>
<td>1.528</td>
</tr>
<tr>
<td>Feed grade salt</td>
<td>0.244</td>
<td>0.227</td>
<td>0.269</td>
</tr>
<tr>
<td>MHA-84%1</td>
<td>0.290</td>
<td>0.349</td>
<td>0.261</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.060</td>
<td>0.063</td>
<td>0.041</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.203</td>
<td>0.175</td>
<td>0.174</td>
</tr>
<tr>
<td>Waldroup vitamins2</td>
<td>0.500</td>
<td>0.500</td>
<td>0.500</td>
</tr>
<tr>
<td>Mintrex P-Se3</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Coban 904</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>BMD-505</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.000</td>
<td>100.000</td>
<td>100.000</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nutrient content</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>21.53</td>
<td>23.91</td>
<td>20.53</td>
<td>22.24</td>
<td>19.31</td>
<td>22.96</td>
</tr>
<tr>
<td>Calcium %</td>
<td>0.90</td>
<td>0.95</td>
<td>0.81</td>
<td>0.86</td>
<td>0.74</td>
<td>0.79</td>
</tr>
<tr>
<td>Nonphytate P, %</td>
<td>0.44</td>
<td>0.47</td>
<td>0.40</td>
<td>0.43</td>
<td>0.036</td>
<td>0.39</td>
</tr>
<tr>
<td>Methionine %</td>
<td>0.61</td>
<td>0.68</td>
<td>0.57</td>
<td>0.62</td>
<td>0.54</td>
<td>0.64</td>
</tr>
<tr>
<td>TSAA %</td>
<td>0.94</td>
<td>1.05</td>
<td>0.90</td>
<td>0.97</td>
<td>0.85</td>
<td>0.99</td>
</tr>
<tr>
<td>Lysine %</td>
<td>1.27</td>
<td>1.42</td>
<td>1.18</td>
<td>1.29</td>
<td>1.11</td>
<td>1.33</td>
</tr>
<tr>
<td>ME kcal/kg</td>
<td>3002.95</td>
<td>3205.71</td>
<td>3025.00</td>
<td>3250.00</td>
<td>3063.54</td>
<td>3250.90</td>
</tr>
</tbody>
</table>

1Methionine hydroxy analogue calcium salt. Novus International, St. Louis, MO 63141

2Provided 7,715 IU of vitamin A (from vitamin A acetate); 5,511 IU of cholecalciferol; 16.53 IU of vitamin E (from dl-alpha-tocopheryl acetate); 0.013 mg of vitamin B12; 6.6 mg of riboflavin; 39 mg of niacin; 10 mg of pantothenic acid; 1.5 mg of menadione (from menadionedimethylpyrimidinol); 0.9 mg of folic acid; 1,000 mg of choline; 1.54 mg of thiamin (from thiamin mononitrate); 2.76 mg of pyridoxine (from pyridoxine HCl); 0.066 mg of d-biotin; and 125 mgethoxyquin per kg of diet.

3Provided 40 mg of Mn (as manganese methionine hydroxy analogue complex); 40 mg of Zn (as zinc methionine hydroxy analogue complex); 20 mg of Cu (as copper methionine hydroxy analogue complex); and 0.3 mg of Se (as selenium yeast) per kg of diet (Novus International, Inc., St. Louis MO).

4Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN 46825

5Bacitracin methylene disalicylate granule, Alpharma Inc., Bridgewater, NJ 08807
Table 2: Live weight, fillet weight and yield, and the frequency of occurrence of three degrees of white striping in breast fillets from broilers fed either a low- (LED) or high-energy diet (HED)

<table>
<thead>
<tr>
<th>Feed treatment</th>
<th>Live weight (g)</th>
<th>Fillet weight (g)(^1)</th>
<th>Fillet yield (%)(^{1,2})</th>
<th>Degree of white striping(^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NORM</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>n</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%</td>
</tr>
<tr>
<td>LED (n = 137)</td>
<td>2787.1b±30.6</td>
<td>260.4b±4.0</td>
<td>9.3a±0.1</td>
<td>65a</td>
</tr>
<tr>
<td>HED (n = 138)</td>
<td>3041.9a±30.5</td>
<td>289.8a±4.0</td>
<td>9.5a±0.1</td>
<td>35b</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Least squares means (± SE) lacking a common superscript within column differ \((P < 0.05)\).

\(^1\) Left fillet (single)

\(^2\) Yield as percentage of live weight

\(^3\) Counts (percentages calculated on row total) of breast fillets with normal (NORM), moderate (MOD), or severe (SEV) occurrence of white striping (Kuttappan et al., 2012).

\(^4\) Fillets showing either MOD or SEV degrees of white striping (WS)
Table 3: Comparison of broiler breast meat from birds with three degrees of white striping

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Degree of white striping</th>
<th>NORM (n=13)</th>
<th>MOD (n=14)</th>
<th>SEV (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live weight (g)</td>
<td></td>
<td>2987.54±78.92</td>
<td>3095.79±71.95</td>
<td>3108.00±103.16</td>
</tr>
<tr>
<td>Fillet weight (g)</td>
<td></td>
<td>266.77±12.60</td>
<td>300.86±10.96</td>
<td>321.88±13.49</td>
</tr>
<tr>
<td>Fillet yield (%)</td>
<td></td>
<td>8.89±0.23</td>
<td>9.71±0.24</td>
<td>10.36±0.26</td>
</tr>
<tr>
<td>Moisture (g/100g)</td>
<td></td>
<td>82.39±0.93</td>
<td>81.30±0.90</td>
<td>81.99±1.20</td>
</tr>
<tr>
<td>Ash (g/100g)</td>
<td></td>
<td>4.58±0.03</td>
<td>4.6±0.03</td>
<td>4.54±0.04</td>
</tr>
<tr>
<td>Fat content (g/100g)</td>
<td></td>
<td>3.03±0.44</td>
<td>4.47±0.42</td>
<td>5.56±0.56</td>
</tr>
<tr>
<td>Calories from fat (kcal/100g)</td>
<td></td>
<td>27.26±3.92</td>
<td>40.2±3.78</td>
<td>50.04±5.00</td>
</tr>
<tr>
<td>Protein content (g/100g)</td>
<td></td>
<td>90.03±0.57</td>
<td>88.93±0.55</td>
<td>87.73±0.73</td>
</tr>
<tr>
<td>Calories from protein (kcal/100g)</td>
<td></td>
<td>360.12±2.28</td>
<td>355.71±2.20</td>
<td>350.9±2.91</td>
</tr>
<tr>
<td>Fat:Protein</td>
<td></td>
<td>0.08±0.01</td>
<td>0.11±0.01</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Total calorie (kcal/100g)</td>
<td></td>
<td>387.38±2.95</td>
<td>395.91±2.85</td>
<td>400.94±3.76</td>
</tr>
</tbody>
</table>

\(^{a,b}\) Least squares means (± SE) with different superscript within row differ \((P < 0.05)\).

\(^1\) Breast fillets with normal (NORM), moderate (MOD), or severe (SEV) occurrence of white striping (Kuttappan et al., 2012).

\(^2\) Left fillet (single)

\(^3\) Yield as percentage of live weight

\(^4\) Dry matter basis

\(^5\) Energy from fat (kcal/100g) = Amount of fat (in g/100g) \(\times 9\)

\(^6\) Energy from protein (kcal/100g) = Amount of protein (in g/100g) \(\times 4\)

\(^7\) Total energy = Calories from fat + calories from protein
Table 4: Fatty acid composition of meat from fillets with normal (NORM) (no striping) and severe (SEV) white striping

<table>
<thead>
<tr>
<th>Fatty acid composition</th>
<th>NORM (n=8)</th>
<th>SEV (n=8)</th>
<th>Pooled SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SFA</td>
<td>32.08</td>
<td>29.98</td>
<td>0.38</td>
<td>**</td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>0.55</td>
<td>0.64</td>
<td>0.03</td>
<td>*</td>
</tr>
<tr>
<td>Pentadecanoic acid (15:0)</td>
<td>0.08</td>
<td>0.14</td>
<td>0.02</td>
<td>*</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>20.7</td>
<td>19.69</td>
<td>0.27</td>
<td>*</td>
</tr>
<tr>
<td>Margaric acid ME (17:0)</td>
<td>0.26</td>
<td>0.27</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Stearic acid (18:0)</td>
<td>10.47</td>
<td>9.15</td>
<td>0.33</td>
<td>*</td>
</tr>
<tr>
<td>Arachidic acid (20:0)</td>
<td>0.02</td>
<td>0.08</td>
<td>0.01</td>
<td>**</td>
</tr>
<tr>
<td>Heneicosanoic acid ME (21:0)</td>
<td>0</td>
<td>0.01</td>
<td>0.00</td>
<td>NS</td>
</tr>
<tr>
<td>MUFA</td>
<td>31.75</td>
<td>34.26</td>
<td>0.80</td>
<td>*</td>
</tr>
<tr>
<td>Myristoleic acid (14:1)</td>
<td>0.03</td>
<td>0.1</td>
<td>0.02</td>
<td>**</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1c)</td>
<td>2.24</td>
<td>2.44</td>
<td>0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Palmitelaidic acid (16:1t)</td>
<td>0.01</td>
<td>0.08</td>
<td>0.01</td>
<td>***</td>
</tr>
<tr>
<td>Vaccenic acid (18:1c11)</td>
<td>2.93</td>
<td>2.7</td>
<td>0.05</td>
<td>**</td>
</tr>
<tr>
<td>Oleic acid (18:1c9)</td>
<td>25.49</td>
<td>27.74</td>
<td>0.67</td>
<td>*</td>
</tr>
<tr>
<td>Elaidic acid (18:1t9)</td>
<td>0.71</td>
<td>0.83</td>
<td>0.02</td>
<td>**</td>
</tr>
<tr>
<td>Gadoleic acid (20:1c11)</td>
<td>0.36</td>
<td>0.36</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>PUFA</td>
<td>33.06</td>
<td>32.74</td>
<td>0.63</td>
<td>NS</td>
</tr>
<tr>
<td>Linoleic acid ME (18:2n-6)</td>
<td>22.3</td>
<td>24.52</td>
<td>0.55</td>
<td>*</td>
</tr>
<tr>
<td>CLA 9-cis, 11-cis (18:2c9c11)</td>
<td>0</td>
<td>0.03</td>
<td>0.01</td>
<td>*</td>
</tr>
<tr>
<td>CLA 9-cis, 11-trans (18:2c9t11)</td>
<td>0</td>
<td>0.07</td>
<td>0.01</td>
<td>***</td>
</tr>
<tr>
<td>α-Linolenic acid (18:3n-3)</td>
<td>1.19</td>
<td>1.59</td>
<td>0.08</td>
<td>**</td>
</tr>
<tr>
<td>γ-Linolenic acid (18:3n-6)</td>
<td>0.13</td>
<td>0.14</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Eicosadienoic acid (20:2n-6)</td>
<td>0.72</td>
<td>0.47</td>
<td>0.05</td>
<td>**</td>
</tr>
<tr>
<td>cis-11,14,17-Eicosatrienoic acid ME (20:3n-3)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.02</td>
<td>NS</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid (20:3n-6)</td>
<td>1.11</td>
<td>0.71</td>
<td>0.07</td>
<td>**</td>
</tr>
<tr>
<td>Arachidonic acid (20:4n-6)</td>
<td>5.55</td>
<td>3.9</td>
<td>0.46</td>
<td>*</td>
</tr>
<tr>
<td>Eicosapentaenoic acid, EPA (20:5n-3)</td>
<td>0.4</td>
<td>0.19</td>
<td>0.04</td>
<td>**</td>
</tr>
<tr>
<td>Docosapentaenoic acid, DPA (22:5n-3)</td>
<td>0.93</td>
<td>0.64</td>
<td>0.08</td>
<td>*</td>
</tr>
<tr>
<td>Docosahexaenoic acid, DHA (22:6n-3)</td>
<td>0.67</td>
<td>0.45</td>
<td>0.06</td>
<td>*</td>
</tr>
<tr>
<td>Other fatty acid peaks</td>
<td>3.11</td>
<td>3.03</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Total fatty acids (mg/g dry sample)</td>
<td>32.16</td>
<td>57.06</td>
<td>4.31</td>
<td>**</td>
</tr>
<tr>
<td>Δ9desaturase (16)2</td>
<td>9.71</td>
<td>11.29</td>
<td>0.67</td>
<td>NS</td>
</tr>
<tr>
<td>Δ9desaturase (18)3</td>
<td>71.37</td>
<td>75.70</td>
<td>1.12</td>
<td>*</td>
</tr>
<tr>
<td>Elongase4</td>
<td>61.51</td>
<td>62.95</td>
<td>0.4</td>
<td>*</td>
</tr>
</tbody>
</table>

1t-trans; c-cis

2Index of Δ9desaturase enzyme activity in 16-carbon fatty acids = ([16:1]/[16:0+16:1])× 100 (Malau-Aduli et al., 1998)
Index of Δ⁶ desaturase enzymes activity in 18-carbon fatty acids = \( \frac{[18:1\text{c9}]}{[18:0] + [18:1\text{c9}]} \times 100 \) (Malau-Aduli et al., 1998)

Index of elongase enzyme activity in the chain lengthening of 16- to 18-carbon fatty acids = \( \frac{[18:0] + [18:1\text{c9}]}{[16:0] + [16:1] + [18:0] + [18:1\text{c9}]} \times 100 \) (Malau-Aduli et al., 1998)

NS = \( P > 0.05 \); \* \( P < 0.05 \); \** \( P < 0.01 \); and \*** \( P < 0.001 \)
Table 5: Comparison of nutritional indices of normal (NORM) and severe (SEV) white striping fillets

<table>
<thead>
<tr>
<th></th>
<th>NORM</th>
<th>SEV</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFA/SFA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.24</td>
<td>2.02</td>
<td>0.04</td>
<td>*</td>
</tr>
<tr>
<td>P/S&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.74</td>
<td>0.89</td>
<td>0.03</td>
<td>**</td>
</tr>
<tr>
<td>n-6/n-3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>19.09</td>
<td>15.60</td>
<td>0.86</td>
<td>*</td>
</tr>
<tr>
<td>Σn-6/Σn-3&lt;sup&gt;4&lt;/sup&gt;</td>
<td>9.2</td>
<td>10.32</td>
<td>0.25</td>
<td>**</td>
</tr>
<tr>
<td>EPA+DHA</td>
<td>1.07</td>
<td>0.64</td>
<td>0.07</td>
<td>**</td>
</tr>
</tbody>
</table>

<sup>1</sup>UFA/SFA = Total MUFA + total PUFA/Total SFA.
<sup>2</sup>P/S = ([18:2n-6] + [18:3n-3]) / ([12:0] + [14:0] + [16:0] + [18:0]) brackets indicate concentrations (Enser et al., 2000).
<sup>3</sup>n-6/n-3 = 18:2n-6/18:3n-3.
<sup>4</sup>Σn-6/Σn-3 = Total (n-6)/Total (n-3).
*P < 0.05 and **P < 0.01
Figure 1: Body weight of birds fed a low- (LED) or high-energy diet (HED) during the starter (0 to 18 d), grower (18 to 32 d), and finisher (32 to 54 d) periods. Within a feeding period, an asterisk (*) indicates a difference ($P < 0.05$) between the two dietary treatments.
Figure 2: Feed conversion ratio (feed: gain) of birds fed a low- (LED) or high-energy diet (HED) during the starter (0 to 18 d), grower (18 to 32 d), finisher (32 to 54 d), and overall (0 to 54 d) feeding periods. Within a feeding period, an asterisk (*) indicates a difference ($P < 0.05$) between the two dietary treatments.
VI.  EFFECT OF DIFFERENT LEVELS OF DIETARY VITAMIN E (DL-α-TOCOPHEROL ACETATE) ON THE OCCURRENCE OF DIFFERENT DEGREES OF WHITE STRIPING ON BROILER BREAST FILLETS

A. ABSTRACT

White striping could be a potential reason for the rejection of raw breast fillets in the market. The condition is characterized grossly by the white striations occurring on the fillets showing dystrophic changes on microscopic examination. Early research have reported similar lesions in case of nutritional muscular dystrophy which is a condition caused mainly by the deficiency of vitamin E in the diet. The present study was intended to evaluate the effect of different levels of dietary vitamin E (DL α-tocopherol acetate) on the incidence of normal (NORM), moderate (MOD) and severe (SEV) degrees of white striping, by modern description, on broiler breast fillets. Basal diet adequate for starter (0 to 18d), grower (19 to 32d) and finisher (33 to 49d) age periods supplemented with 15, 50, 100, 200 and 400 mg of vitamin E/kg of feed were used in the study. Each of the five diet treatments were fed to eight pens (53 birds each) of male broilers from a commercial strain. At 49 d, five birds were randomly selected from each pen (n = 40 birds/diet treatment) and were processed. Live weight, ready-to-cook (RTC) weight, weight of the fillets, wings, tenders, legs and the racks were obtained. The fillets were scored for the three degrees of white striping. There were no significant differences among the diet treatments with respect to the weight and carcass yield parameters. Furthermore, the diet treatments did not show any significant effect on the occurrence of NORM, MOD and SEV degrees of white striping. However, the fillet weight was the only parameter which had a significant effect on the occurrence of white striping. Higher degrees of white striping were seen associated with heavier fillets, which is in accordance with previous studies. Different levels of
vitamin E levels used in the present study did not show any significant effect on the occurrence of three degrees of white striping. These results suggest that dietary vitamin E level is not associated with the modern condition of white striping in broiler breast meat.

B. INTRODUCTION

Vitamin E is a fat soluble vitamin which is of plant origin. It is important for the health of both humans and animals due to its effect on the integrity and optimal function of various organ systems. Most important property of vitamin E is that it can act as a lipid soluble antioxidant. It acts synergistically with selenium dependent - glutathione peroxidase, catalase and superoxide dismutase to remove the intermediates formed during the free radical chain reaction (Herrera and Barbas, 2001). Thus, it will help to prevent the propagation of free radicals and avoid the damages to DNA, proteins (including enzymes), and the polyunsaturated fatty acids present on cell membrane (Mezes et al., 1997). Besides this, vitamin E has immunoregulatory effect which could be through the modulation of cyclooxygenase and lipoxygenase pathways resulting in control on the synthesis of substances like prostaglandins and leukotrienes (Blumberg, 1994; Leshchinsky and Klasing, 2001).

According to National Research Council (1994) requirements, the dietary requirement of vitamin E for broiler is 10 IU/kg of feed. However, establishment of vitamin E requirements in isolation is difficult because of its interrelationships with various other conditions. Hidiroglou et al. (1992) suggested that the requirement of vitamin E may be influenced by various factors affecting the variability of vitamin E in feedstuffs and the physiological status of the bird. Some of these factors are the amount, type and degree of oxidation of fat present in the diet, presence of other dietary antioxidants like selenium, iron, copper, sulfur containing amino acids etc., and
harvesting, drying or storage conditions of feeds that results in destruction of vitamin E. It is also necessary to consider the possible genetic differences in requirements, variations in absorbability of vitamin E, destruction of vitamin E in the gastrointestinal tract, variation in the quantity of vitamin E transferred from breeder hen to chick, increased requirements due to diseases, stress or increased metabolic demands (Hidiroglou et al., 1992).

Clinical manifestations of the deficiency of vitamin E may depend upon the species of the animal or bird, age and also the organs affected. The deficiency of vitamin E, along with associated nutrients like selenium and sulfur containing amino acids, can mainly result in pathological condition such as encephalomalacia, exudative diathesis, nutritional muscular dystrophy (NMD) in chicks, ducks and turkeys (Klasing, 2008). Nutritional muscular dystrophy is characterized grossly by white striations in the breast muscle in the direction of the muscle fibers and sometimes also in the leg muscles (Dam et al., 1952; Scott et al., 1955; Machlin and Shalkop, 1956; Machlin and Pearson, 1956; Nesheim et al., 1959; Ferguson et al., 1964; Bunyan et al., 1967; Netke et al., 1969; Klasing, 2008). There will be wide linear areas of degeneration involving several adjacent muscle fasiculi which contribute to the white colored bands seen grossly. The microscopic examination of the lesions showed hyaline, waxy or Zenker’s degeneration accompanied with fragmentation, hyalinization, loss of striation, multiplication of cells, infiltration by heterophil cells, clumping of fibers into eosinophilic masses and in more severe cases with calcification was well (Dam et al., 1952; Machlin and Shalkop, 1956).

Recently, a condition known as white striping, which has a negative impact on the consumer acceptance of broiler breast fillets (Kuttappan et al., 2012a) is gaining the attention of broiler producers. Gross and microscopic lesions associated with white striping are similar to those of nutritional muscular dystrophy previously described (Kuttappan et al., 2009; Kuttappan
et al., 2011). However, the occurrence of white striping is seen in birds which are fed with adequate amount of Vitamin E in their diets. Perhaps, the fast growing birds may need greater amounts of vitamin E in their muscles for a normal muscle growth. Various studies showed that the increase in dietary level of vitamin E could result in increased amount of the vitamin E in muscles (Sheldon, 1984; Sheehy et al., 1991; De Winne and Dirinck, 1996; Lauridsen et al., 1997). Therefore, the present study is intended to compare the effect of different levels of dietary vitamin E on the occurrence of three degrees of white striping in broiler breast fillets.

C. MATERIALS AND METHODS

Experimental design

The present study included five different diet treatments with 15, 50, 100, 200 and 400 IU/kg of vitamin E levels. The basal diet was formulated in such a way as to meet the typical industry nutrient standards (Agri-Stats, Fort Wayne, IN). The composition of the basal diet for the starter (1 to 18 d), grower (19 to 32 d) and finisher (33 to 49 d) age periods of birds were as given in the Table 1 and 2. Diet also contained phytase (equivalent of 0.10% nPP and 0.10% Ca) and was fortified with complete vitamin (without vitamin E) and mineral mixture. The basal diet was then supplemented with different levels (15, 50, 100, 200 and 400 mg/kg) of DL-\(\alpha\)-tocopherol acetate as a source of vitamin E. Each of the five diet treatments were assigned to male broilers of a commercial strain in a completely randomized design. There were eight replicates, with 53 birds per replicate. The birds were maintained on litter floor pens (1.5 X 3 m) with \textit{ad libitum} access to feed and water. At 49 d, five birds were randomly selected from each pen and were processed using commercial style methods.
**Processing of the birds**

Feed was withdrawn 8h before processing, while the birds were given free access to water. All the birds were weighed and processed in the commercial inline system at the University of Arkansas Processing Pilot Plant on the same day. At the plant, the birds were hung on a shackle line, electrically stunned (11V, 11mA, 11s), manually severed the left carotid artery and jugular vein, bled out (1.5 min), soft scalded (53.8°C, 2min) and picked using inline commercial defeathering equipment. The carcasses were then eviscerated, rinsed, prechilled at 12°C for 15min and chilled for 45min at 1°C in immersion chilling tanks. Each carcass was weighed separately before deboning and the Ready-to-cook (RTC) weight was obtained. After deboning, weight of the breast fillets, wings, tenders, legs and the racks were obtained for each carcass. The yield of carcass parts were calculated and reported both as the percentage of live and RTC weights. All the fillets (pectoralis major) were scored to estimate the frequency of three degrees (NORM, MOD and SEV) of white striping as described by Kuttappan et al. (2012a).

**Statistical analysis**

Live weight, RTC weight, weight of the carcass parts and the yield (both based on live weight and RTC weight) data were analyzed using GLM and LS means were calculated and separated with Tukey’s HSD test at $P < 0.05$ (JMP version 9.0, SAS Institute Inc.). The parameters associated with the occurrence of three degrees of white striping were determined using multinomial stepwise logistic regression (PASW Statistics 18, SPSS Inc., Chicago, IL, USA). During the above analysis, the MOD and SEV degrees of white striping were considered as the dependent variables with reference to NORM category. The independent variables were the diet treatments, mean centered (to avoid multicollinearity) covariates (live weight, RTC
weight, weight of the carcass parts and the yields, both based on live weight and RTC weight) and their two and three way interactions. The results from the analysis were reported as the independents variable/s included in the model which has/have a significant ($P < 0.05$) effect on the occurrence of white striping. Odd ratio, 95 % confidence interval (CI) and the respective $P$ values for the independent variable(s) included in the model were obtained to evaluate the pattern of association. Additionally, in order to evaluate the pattern of occurrence of three degrees of white striping with respect to the factors included in the final model, the data was grouped into equal sized bins using interactive binning procedure with cut off set at 10th percentile (JMP 9.0, SAS Institute Inc.). The mean estimated probabilities were then calculated and plotted to the respective bins.

D. RESULTS AND DISCUSSION

Weights and yields of broilers fed varying levels of vitamin E are shown in Table 3. In the present study, there were no significant differences in any of these weights or yield parameters with respect to the level of dietary vitamin E. This finding was in agreement with previous studies conducted where body weights were not affected by feeding different levels of dietary $\alpha$-tocopherol (Sheehy et al., 1991; Bartov and Frigg, 1992). Meanwhile, Chae et al. (2006) reported that 100 and 200 mg of $\alpha$-tocopherol acetate/kg of feed resulted in significant improvement in weight gain when compared to the negative control group. However, they did not observe any significant differences in the dressing and the breast meat percentages. Gao et al. (2010) reported that higher levels of $\alpha$-tocopherol acetate (200 IU/kg of feed) could alleviate the oxidative stress produced by dexamethasone injection and can improve the growth performance in broiler chickens. Rice and Kennedy (1988) suggested that the advantage of higher levels of dietary vitamin E will be witnessed mainly when the group is exposed to some kind of stressors.
The absence of differences in the growth performance among the five dietary treatments in this study indicates that all the birds might have been exposed to stress below the threshold level to get a beneficiary effect from higher levels of dietary vitamin E on growth performance and yield.

The distributions of three degrees of white striping in the diet treatment groups are shown in Table 4. Some variation was seen in the frequency but no consistent trend was observed. The majority (66 to 86%) of birds in every treatment exhibited MOD striping. Furthermore, there was no evidence to indicate that higher levels of dietary vitamin E would decrease the incidence of white striping. This was supported by the findings from multinomial regression analysis. The results from the logistic regression revealed that among the different variables (diet treatments, live weight, RTC weight, weight of the carcass parts and the yield and their two and three way interactions), only fillet weight was significantly ($P < 0.05$) associated with the occurrence of three degrees of white striping (Table 5). It was determined based on the OR of fillet weight in relation to the occurrence of three degrees of white striping. Odds ratio (OR) indicates the increased or decreased chance of occurrence of a dependent category (MOD and SEV degrees of white striping) as a result of unit increase in the continuous variable (fillet weight in g). An OR $> 1$ indicates an increased chance while an OR $< 1$ indicates lesser chance of occurrence. The results from the present study showed that both MOD and SEV had an OR greater than one which implies that with reference to NORM there was a higher chance of occurrence of MOD and SEV fillets as the fillet weight increased. Therefore, MOD and SEV degrees of white striping are seen associated with heavier fillets. The finding was in accordance with results from previous studies (Bauermeister et al., 2009; Kuttappan et al., 2009).

The pattern of occurrence of three degrees of white striping with respect to the fillet weight is shown in Figure 1. The graph clearly indicates that fillet weight does have an effect. As
the fillet weight increased, there was a reduction in the probability of occurrence of NORM fillets while that of SEV fillets increased. MOD fillets showed a peak where the lines of NORM and SEV crossed and it decreased towards either side where there was an increased probability for either the NORM or the SEV fillets. However, the present study showed a higher percentage (85.45%) of fillets with white striping (either the MOD or SEV) while the percentage reported by Kuttappan et al. (2009) was 55.75%. Here, the chance for individual variations in scoring is less because same people did the scoring for both of these studies. Interestingly, the birds used in the present study had a higher mean RTC weight (2856.26 g) at a younger age (49 d) than those used in the previous study (RTC wt 2440.01 g; 59 to 63 d age), which could have ensued in higher incidence of white striping due to increased growth rate. In fact, Kuttappan et al. (2012b) reported that increased growth rate resulted in increased incidence of higher degrees of white striping. Further studies are warranted to determine the incidence of the condition in the poultry meat industry.

These results suggest that there could be differences in the etiology of NMD and the recently observed condition of white striping. Even though the main gross lesion associated with white striping seems to be similar to NMD, the pattern of the lesion may be different. White striping shows clear white lines on the surface of normal colored fillets, while NMD lesions are seen as coalescing white areas and the fillets are pale in color. Another important aspect is that the poultry diet low in vitamin E may also be associated with other conditions like encephalomalacia and exudative diathesis (Klasing, 2008). However, such pathological conditions have not been observed in flocks showing the occurrence of white striping. So far, occurrence of higher degrees of white striping can be seen even in birds fed with adequate amounts of dietary vitamin E. In addition, the occurrence of normal, moderate and severe lesions
of white striping can be seen in flocks grown on the same diet and the more severe cases are associated with heavier and older birds (Bauermeister et al., 2009; Kuttappan et al., 2009). These results imply that the occurrence of white striping may not be directly associated with a dietary deficiency of vitamin E or the associated nutrients.

Based on the studies conducted so far, white striping occurs due to muscle damage mainly associated with higher growth rate in broiler birds (Kuttappan et al., 2009, 2012b). In other words, white striping could be a growth associated myopathic condition. However, the lack of effect due to increased dietary vitamin E does not necessarily confirm that vitamin E is ineffective. There may be conditions which reduce or prevent the amount of active vitamin E reaching the breast muscle. Previous research has suggested that muscle fiber growth in rapidly growing birds may have outgrown their supporting systems (Wilson et al., 1990), especially decreased capillary density which could result in reduced supply of nutrients and oxygen and also slower removal of lactic acid from the muscles (Hoving-Bolink et al., 2000), leading to dystrophic changes. Even though the increased dietary vitamin E can increase the level of vitamin E in muscle, it will mainly depend on the vascularity to the muscle tissue (Sheldon, 1984; De Winne and Dirinck, 1996). Further studies are needed to evaluate the capillary density in relation to the amount of Vitamin E in the breast muscles in fast growing broilers. The results from the present study suggests that even if there is reduction in the vitamin E reaching the fast growing muscles, we may not be able to compensate it with increasing dietary vitamin E.

In conclusion, though white striping has some similarities to NMD caused by the deficiency of dietary vitamin E, the increments of dietary vitamin E (15, 50, 100, 200 and 400 IU/kg of fed) used in the study did not result in any significant association with respect to the occurrence of white striping in broiler breast fillets. This implies that, in contrast to NMD, an
increased level of dietary vitamin E cannot prevent the occurrence of white striping. Meanwhile, the occurrence of white striping is significantly related to the weight of the fillets, when compared to other carcass weight and yield parameters evaluated in the study.
E. REFERENCES


Table 1: Composition (% as fed) and the calculated nutrient content of basal diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Starter (1-18 d)</th>
<th>Grower (19-32 d)</th>
<th>Finisher (33-49 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow corn</td>
<td>57.822</td>
<td>62.528</td>
<td>68.0500</td>
</tr>
<tr>
<td>Poultry oil</td>
<td>1.553</td>
<td>2.297</td>
<td>2.622</td>
</tr>
<tr>
<td>Meat and bone meal</td>
<td>4.00</td>
<td>3.00</td>
<td>2.500</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>34.84</td>
<td>30.265</td>
<td>24.923</td>
</tr>
<tr>
<td>Limestone</td>
<td>0.421</td>
<td>0.500</td>
<td>0.577</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.238</td>
<td>0.281</td>
<td>0.230</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>0.373</td>
<td>0.407</td>
<td>0.430</td>
</tr>
<tr>
<td>MHA-84(^1)</td>
<td>0.369</td>
<td>0.332</td>
<td>0.287</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>0.014</td>
<td>0.014</td>
<td>0.017</td>
</tr>
<tr>
<td>L-Lysine HCl</td>
<td>0.101</td>
<td>0.117</td>
<td>0.113</td>
</tr>
<tr>
<td>Vitamin premix(^2)</td>
<td>0.050</td>
<td>0.040</td>
<td>0.032</td>
</tr>
<tr>
<td>Trace mineral mix(^3)</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Coban 90(^4)</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>DSM phytase</td>
<td>0.019</td>
<td>0.019</td>
<td>0.019</td>
</tr>
<tr>
<td>Selenium 0.06% premix</td>
<td>0.050</td>
<td>0.050</td>
<td>0.050</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.000</td>
<td>100.000</td>
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</table>

Nutrient content

<table>
<thead>
<tr>
<th></th>
<th>Starter (1-18 d)</th>
<th>Grower (19-32 d)</th>
<th>Finisher (33-49 d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>22.75</td>
<td>20.48</td>
<td>18.14</td>
</tr>
<tr>
<td>Calcium, %(^5)</td>
<td>0.91</td>
<td>0.83</td>
<td>0.77</td>
</tr>
<tr>
<td>Total P, %</td>
<td>0.63</td>
<td>0.57</td>
<td>0.51</td>
</tr>
<tr>
<td>Available P, %(^5)</td>
<td>0.46</td>
<td>0.41</td>
<td>0.37</td>
</tr>
<tr>
<td>Methionine, %</td>
<td>0.64</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>Lysine, %</td>
<td>1.32</td>
<td>1.19</td>
<td>1.03</td>
</tr>
<tr>
<td>Tryptophan, %</td>
<td>0.27</td>
<td>0.24</td>
<td>0.21</td>
</tr>
<tr>
<td>Threonine, %</td>
<td>0.89</td>
<td>0.80</td>
<td>0.71</td>
</tr>
<tr>
<td>Arginine, %</td>
<td>1.53</td>
<td>1.36</td>
<td>1.17</td>
</tr>
<tr>
<td>Sodium, %</td>
<td>0.20</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>TSAA, %</td>
<td>1.00</td>
<td>0.91</td>
<td>0.82</td>
</tr>
<tr>
<td>ME, kcal/lb</td>
<td>1376.00</td>
<td>1412.00</td>
<td>1441.00</td>
</tr>
</tbody>
</table>

\(^1\) Methionine hydroxyl analogue calcium salt. Novus International, St. Louis, MO
\(^2\) See Table 2 for details
\(^3\) Provides per kg of diet: Mn (from MnSO\(_4\).H\(_2\)O) 100mg; Zn (from ZnSO\(_4\).7H\(_2\)O) 100mg; Fe (from FeSO\(_4\).7H\(_2\)O) 50mg; Cu (from CuSO\(_4\).5H\(_2\)O) 10mg; I from Ca(IO\(_3\))\(_2\).H\(_2\)O, 1.0mg.
\(^4\)Elanco Animal Health division of Eli Lilly & Co., Indianapolis, IN 46825
\(^5\) Assumes 0.10% equivalency from phytase addition
<table>
<thead>
<tr>
<th></th>
<th>Starter (1-18 d)</th>
<th>Grower (19-32 d)</th>
<th>Finisher (33-49 d)</th>
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<tbody>
<tr>
<td>Vitamin A, IU</td>
<td>9,300</td>
<td>7,440</td>
<td>5,952</td>
</tr>
<tr>
<td>Vitamin D, IU</td>
<td>3,110</td>
<td>2,488</td>
<td>1,990</td>
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<tr>
<td>Vitamin K, mg</td>
<td>1.9</td>
<td>1.5</td>
<td>1.2</td>
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<tr>
<td>Niacin, mg</td>
<td>48.2</td>
<td>38.6</td>
<td>30.8</td>
</tr>
<tr>
<td>Pantothenic, mg</td>
<td>13.4</td>
<td>10.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Riboflavin, mg</td>
<td>8.7</td>
<td>7.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Thiamin, mg</td>
<td>2.1</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Pyridoxine, mg</td>
<td>2.9</td>
<td>2.3</td>
<td>1.9</td>
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<tr>
<td>Folic, mg</td>
<td>0.955</td>
<td>0.764</td>
<td>0.611</td>
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<tr>
<td>Biotin, mg</td>
<td>0.101</td>
<td>0.081</td>
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</tr>
<tr>
<td>Vitamin B12, mg</td>
<td>0.0145</td>
<td>0.012</td>
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Table 3: Mean weights and yields of broilers fed various levels of vitamin E

<table>
<thead>
<tr>
<th>Parameter</th>
<th>15 IU/kg</th>
<th>50 IU/kg</th>
<th>100 IU/kg</th>
<th>200 IU/kg</th>
<th>400 IU/kg</th>
<th>Pooled SEM</th>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Live</td>
<td>3517</td>
<td>3561</td>
<td>3545</td>
<td>3627</td>
<td>3595</td>
<td>36.35</td>
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<tr>
<td>RTC2</td>
<td>2782</td>
<td>2863</td>
<td>2827</td>
<td>2866</td>
<td>2851</td>
<td>33.98</td>
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<tr>
<td>Wings</td>
<td>286</td>
<td>287</td>
<td>289</td>
<td>291</td>
<td>298</td>
<td>3.37</td>
</tr>
<tr>
<td>Breast</td>
<td>659</td>
<td>679</td>
<td>688</td>
<td>691</td>
<td>696</td>
<td>13.01</td>
</tr>
<tr>
<td>Tenders</td>
<td>139</td>
<td>147</td>
<td>144</td>
<td>146</td>
<td>148</td>
<td>2.30</td>
</tr>
<tr>
<td>Legs</td>
<td>863</td>
<td>900</td>
<td>870</td>
<td>899</td>
<td>894</td>
<td>10.40</td>
</tr>
<tr>
<td>Rack</td>
<td>800</td>
<td>816</td>
<td>810</td>
<td>806</td>
<td>810</td>
<td>10.87</td>
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</table>

<table>
<thead>
<tr>
<th>Yield (% of live weight)</th>
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<th></th>
<th></th>
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<tr>
<td>RTC</td>
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<td>80.3</td>
<td>80.0</td>
<td>79.0</td>
<td>79.3</td>
<td>0.42</td>
</tr>
<tr>
<td>Wings</td>
<td>8.2</td>
<td>8.1</td>
<td>8.2</td>
<td>8.0</td>
<td>8.2</td>
<td>0.08</td>
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<tr>
<td>Breast</td>
<td>18.8</td>
<td>19.0</td>
<td>19.4</td>
<td>19.0</td>
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<td>0.28</td>
</tr>
<tr>
<td>Tenders</td>
<td>4.0</td>
<td>4.1</td>
<td>4.1</td>
<td>4.0</td>
<td>4.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Leg</td>
<td>24.7</td>
<td>25.2</td>
<td>24.6</td>
<td>24.7</td>
<td>24.6</td>
<td>0.24</td>
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<tr>
<td>Rack</td>
<td>22.8</td>
<td>22.9</td>
<td>22.9</td>
<td>22.3</td>
<td>22.4</td>
<td>0.22</td>
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</table>

<table>
<thead>
<tr>
<th>Yield (% of RTC weight)</th>
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<th></th>
<th></th>
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<th></th>
<th></th>
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<tr>
<td>Wings</td>
<td>10.3</td>
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<td>10.3</td>
<td>10.2</td>
<td>10.3</td>
<td>0.10</td>
</tr>
<tr>
<td>Breast</td>
<td>23.6</td>
<td>23.7</td>
<td>24.2</td>
<td>24.1</td>
<td>24.2</td>
<td>0.32</td>
</tr>
<tr>
<td>Tenders</td>
<td>5.0</td>
<td>5.2</td>
<td>5.1</td>
<td>5.1</td>
<td>5.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Legs</td>
<td>31.2</td>
<td>31.3</td>
<td>30.8</td>
<td>31.3</td>
<td>31.2</td>
<td>0.26</td>
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<tr>
<td>Rack</td>
<td>28.8</td>
<td>28.5</td>
<td>28.6</td>
<td>28.3</td>
<td>28.2</td>
<td>0.24</td>
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</table>

1No significant differences noted among treatments ($P > 0.05$)

2RTC = Ready to cook carcass
Table 4: Frequency of occurrence of three degrees of white striping in five diet treatments

<table>
<thead>
<tr>
<th>Dietary vitamin E level</th>
<th>NORM</th>
<th>MOD</th>
<th>SEV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Count</td>
<td>%</td>
<td>Count</td>
</tr>
<tr>
<td>15 IU/kg(n=35)</td>
<td>8</td>
<td>22.86</td>
<td>27</td>
</tr>
<tr>
<td>50 IU/kg(n=33)</td>
<td>7</td>
<td>21.21</td>
<td>22</td>
</tr>
<tr>
<td>100 IU/kg(n=36)</td>
<td>3</td>
<td>8.33</td>
<td>32</td>
</tr>
<tr>
<td>200 IU/kg(n=38)</td>
<td>6</td>
<td>15.79</td>
<td>29</td>
</tr>
<tr>
<td>400 IU/kg(n=38)</td>
<td>2</td>
<td>5.26</td>
<td>33</td>
</tr>
</tbody>
</table>

1Breast fillets with normal or no striping (NORM), moderate (MOD), or severe (SEV) degree of white striping.
Table 5: Odd ratio (OR), 95% confidence interval (CI) and the probability level of the variable associated with the occurrence of moderate (MOD) and severe (SEV) degrees of white striping

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MOD</th>
<th></th>
<th></th>
<th>SEV</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
<td>P value</td>
<td>OR</td>
<td>95% CI</td>
<td>P value</td>
</tr>
<tr>
<td>Fillet weight</td>
<td>1.011</td>
<td>1.003-1.018</td>
<td>0.004</td>
<td>1.026</td>
<td>1.013-1.039</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

1Odd ratio determined with the NORM (no striping) category as the reference.
Figure 1: Probability of occurrence of white striping with respect to the weight of the butterfly breast fillets. NORM = normal fillets/no visual white striping, MOD = moderate degree of white striping, SEV = severe degree of white striping. Values were pooled across the diet treatments.
VII. PATHOLOGICAL CHANGES ASSOCIATED WITH WHITE STRIPING IN BROILER BREAST MUSCLES

A. ABSTRACT

White striping is a condition in broiler chickens characterized grossly by the occurrence of white striations, seen parallel to the direction of muscle fibers, on broiler breast fillets and thighs. Based on visual evaluation of the intensity of white striping, breast fillets can be categorized into normal (NORM), moderate (MOD) and severe (SEV) categories. A detailed microscopic evaluation of the fillet samples could provide information regarding the structural and compositional changes associated with the occurrence of white striping. The present study was undertaken to evaluate the details of histological changes occurring in the fillets with respect to the three degrees of white striping. Representative breast fillets, for each degree of white striping (n = 20), were collected from 45-d-old broilers, approximately 2 h post mortem (PM). From each fillet, two skeletal muscle samples were obtained and fixed in 10% neutral buffered formalin. To identify and differentiate the histological changes, slides were prepared and stained using Hematoxylin and eosin, Masson’s Trichrome, and Oil Red O stains. Major changes observed in the MOD and SEV samples consisted of loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis, and interstitial inflammation and fibrosis. Microscopic lesions were visually scored mainly for degeneration and necrosis, fibrosis and lipidosis in the samples. The scale used to score the samples ranged from 0 (normal) to 3 (severe). There was a significant ($P < 0.05$) increase in mean scores for degenerative or necrotic lesions, fibrosis, and lipidosis as the degree of white striping increased from NORM to SEV. The histopathological findings from the present study
show that the changes occurring in white striping are a degenerative myopathy of unknown cause.

B. INTRODUCTION

Visual appearance is the single meat quality which decides the purchase of a packed meat product in a grocery store. Any deviation from the normal appearance will result in the rejection of the product in meat market, irrespective of the other superior qualities. Recently, Kuttappan et al. (2012a) reported that white striping is a condition which could reduce the consumer acceptance and purchase intent for boneless skinless broiler breast fillets. The condition is characterized by white striation seen parallel to the direction of muscle fibers in broiler breast fillets, and there can be varying severity. Based on that, fillets can be classified as normal (NORM), moderate (MOD) and severe (SEV) (Kuttappan et al., 2012a). White striping is associated with heavier body weight or enhanced growth rate in birds (Bauermeister et al., 2009; Kuttappan et al., 2012b). Kuttappan et al. (2012c) also reported that the occurrence of SEV degree of white striping is associated with higher fat content in broiler breast fillets of birds from the same strain fed with same diet formulation. Currently, producers are concerned about the influence of white striping on the poultry meat market. However, little is known about the tissue changes associated with the occurrence of the condition in broilers and the possible etiology.

Gross, microscopic and subcellular properties of skeletal muscle may vary depending on the species, strains, anatomical location, stage of development or even the different portions within the same muscle (Mahon et al., 1984; Mahon, 1999). Also, the appearance of white regions in meat will give a primary impression of marbling to the consumer, as in the case of beef or pork. This was reflected in case of white striping evaluated by consumers (Kuttappan et al., 2012a). Alternatively, some of the earlier studies reported that the occurrence of white
striations on broiler breast fillets was due to conditions like hereditary muscular dystrophy
(Asmundson and Julian, 1956; Julian and Asmundson, 1963) and nutritional myopathy due to the
deficiency of vitamin E and associated nutrients (Dam et al., 1952; Machlin and Shalkop, 1956).
In fact, the occurrence of pale streaks may be a manifestation of mineralization or infiltration of
collagen or fat as a sequel to myofiber necrosis due to any cause (Valentine and McGavin, 2012).
According to Valentine and McGavin (2012), the cytoarchitectural characteristics of skeletal
muscle are best evaluated by microscopic examination. Furthermore, the comparison of
microscopic lesions of white striping with those of various muscular abnormalities in poultry
could help to make meaningful conclusions about the etiology of the condition. We hypothesize
that there may be differences in microscopic lesions between the NORM, MOD and SEV
degrees of white striping which could have resulted in a change in proximate composition
reported by (Kuttappan et al., 2012b). The purpose of this study was to identify, quantify and
compare the histological lesions seen in broiler breast fillets with NORM, MOD and SEV
degrees of white striping. Also, comparison was made in lesions seen on different portions
within the breast fillets as well as various other muscles. The proximate composition of breast
fillets with NORM, MOD and SEV degrees was also determined to support the histological
findings. Furthermore, attempts were made to compare and contrast the lesions of white striping
and various muscle abnormalities in poultry and to derive speculations about the etiology.

C. MATERIALS AND METHODS

Experiment 1

Muscle samples for the histologic study were collected from birds processed at 45d of
age. The samples used in the present study were collected from birds (market age) processed
under the commercial-style inline processing system (Mehaffey et al., 2006) at University of Arkansas Poultry Processing Pilot Plant. All the birds were withdrawn from feed for 10h prior to slaughter, but were given free access to water. During processing, the birds were electrically stunned, exsanguinated by severing the left carotid and jugular vein, soft scalded, defeathered and manually eviscerated. The carcasses were hot deboned (without chilling) at 30 min post mortem (PM). Since the condition is present at the time of processing, carcasses were hot deboned so that samples for the histology could be collected with minimal autolytic changes. The left breast fillet from each bird was screened to determine the degree of white striping (Kuttappan et al., 2012a). Briefly, the fillets were categorized as NORM, MOD and SEV based on the size and distribution of the gross white striations on the surface. Fillets with apparently no white striations were considered to be normal (NORM). Those fillets with white striations generally < 1mm thick, but readily observed on the fillet surface were considered to be moderate (MOD) degree of white striping. The severe (SEV) category of fillets had thick white striations (generally > 1mm thick) which covered a greater area of the fillet surface. After scoring for the degree of white striping, the muscle samples were collected from the cranial region on the ventral surface or from the skin side of the left-side breast fillets (pectoralis major) of 60 birds (n = 20/each degree of white striping). From another 18 birds (n = 6/each degree of white striping), samples were collected from the ventral and dorsal (bone-side) surfaces of the cranial region of breast fillets (pectoralis major), tenders (pectoralis minor), thighs (iliotibialis) and drumsticks (gastrocnemius) of each bird to compare the changes occurring in other muscles (see the appendix). Two muscle sections were collected from each muscle. Special attention was given to keep the sampling site consistent throughout the study. All the muscle sections were cut along the direction of muscle fibers and fixed in 10% buffered neutral formalin. Later, the tissues were
embedded in paraffin, sectioned at 4μm and stained with hematoxylin and eosin (H & E) stain. Three tissue sections were prepared from each muscle sample collected. The H & E stained slides were mainly used to evaluate the myopathic lesions in the tissue. The muscle tissues were also stained with Oil Red O and Masson’s Trichrome stains to confirm the presence of lipid and collagen, respectively. The Oil Red O staining is specific for fat, where fat is shown in red and the nuclei are blue (Jones, 2002a). Masson’s Trichrome stains the muscle, collagen and nuclei with red, blue and blue-black, respectively (Jones, 2002b). The degree of myopathic lesions, fibrosis and lipidosis were quantified by visually scoring the tissue slides under bright field microscopy. The histopathological score was based on a scale ranging from 0 to 3, with normal (0), mild (1), moderate (2) and severe (3) (Figure 1). The histological lesions were evaluated separately by four veterinary pathologists while the scoring of histopathological lesions was performed at California Animal Health and Food Safety Laboratory, Tulare, California.

**Experiment 2**

Muscle samples for proximate analysis were collected from 45 birds (n = 15/each degree of white striping), of 57 d old. The birds were processed and the fillets were scored for the degree of white striping as explained in Experiment 1. The left-side breast fillets were longitudinally split into equal halves of dorsal and ventral sections. About 50 g of meat was collected from the cranial end of the dorsal and ventral halves of each fillet. Proximate composition of the raw breast fillets was determined at the University of Arkansas Central Analytical Laboratory. Breast fillet samples were weighed in plastic containers and placed in a freeze-dryer (Virtis Genesis, Gardiner, NY) set at -10°C, and were allowed to freeze-dry for 8 d until the pressure reached 0 mm of mercury. Fat and protein contents of the freeze-dried samples
were estimated using ether extraction (AOAC #920.39C) and combustion (AOAC #990.03) methods, respectively (AOAC, 1990), and were reported as percentages on a DM basis.

**Statistical analysis**

The data were analyzed using ANOVA (SAS, Institute, Inc.). The means were separated with Tukey’s test with $P < 0.05$ considered as significant. The myopathic lesions, fibrosis and lipidosis scores as well as the proximate composition of the muscle samples were analyzed to evaluate the effect of NORM, MOD and SEV degrees of white striping. The effect of location (dorsal or ventral portions) on the proximate composition of each degree of white striping was determined using Student’s t test at $P < 0.05$. Individual birds were considered as the experimental unit for the entire analysis.

**D. RESULTS**

In the present study, gross lesions on the dorsal and ventral surfaces of the fillets (pectoralis major), as well as various other muscles such as tenders (pectoralis minor), thighs (iliotibialis) and drumsticks (gastrocnemius) were compared. Within the same fillet, it was found that the severity of the condition was greater towards the cranial end where the fillet was thicker compared to the caudal portion. Even though the ventral (skin-side) surface of MOD and SEV fillets showed distinct lines, the gross lesions were comparatively less on the dorsal (bone-side) portion. However, white flecks of varying size were seen throughout the fillet. Furthermore, the occurrence of white striations was more distinct on fillets and thighs muscles while it was less apparent on tenders and drumsticks. The histopathological analysis showed profound degenerative myopathic lesions along with replacement of chronically damaged muscle with adipocytes and fibrosis in the muscle tissue with a higher degree of white striping. The
microscopic lesions included floccular/vacuolar degeneration, lysis, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells) and interstitial inflammation along with fibrosis. There were multiple rounded hypereosinophilic fibers with loss of cross striation and internalization of nuclei. The interstitium showed multifocal edema with infiltration by lymphocytes and macrophages. There were several muscle fibers which were fragmented and undergoing phagocytosis. The muscle cells seemed to have variability in fiber size even though it was difficult to confirm that in the face of degeneration and regeneration. The polyphasic (both acute and chronic) changes in the same sections suggested continuous and ongoing exposure to the causative insult (Valentine and McGavin, 2012). Increased severity (MOD and SEV) of the white striping was related to increased chronicity of myopathic lesions although the NORM muscle occasionally showed hypereosinophilic fibers with loss of cross striations, internalization of nuclei and few infiltrates of lymphocytes and macrophages. The analysis of the histopathological score revealed that as the degree of white striping increased, there was a significant \( P < 0.05 \) increase in the occurrence of chronic myopathic lesions along with lipidosis and fibrosis (Figure 2). Furthermore, the histopathological score from different muscle samples showed that the fillets (ventral surface) and thigh muscle had a significant \( P < 0.05 \) increase in the myopathic lesions with respect to an increase in the degree of gross lesions of the breast fillets (Table 1). This histopathological observation was in accordance with the gross lesions observed in these muscle samples.

The results from the proximate analysis of both the dorsal and ventral surface samples from the broiler breast fillets with different degrees of white striping were in concurrence with the histopathological observations. The samples collected from the ventral surface of the fillets showed an increase \( P < 0.05 \) in the fat content (% of DM) as the degree of white striping
increased (Figure 3). Interestingly, there was no \((P > 0.05)\) difference in the fat content in the meat samples collected from ventral surface of MOD and SEV, although these two had a higher \((P < 0.05)\) fat content when compared to NORM. Furthermore, the ventral surfaces of the MOD and SEV fillets had a higher \((P < 0.05)\) fat content than the corresponding dorsal surfaces, while there was no difference in fat content due to location within the NORM fillets. In addition, as the fat content increased associated with increase in the degree of white striping, there was a corresponding decrease in protein content. The dorsal and ventral surfaces on the NORM fillet had higher \((P < 0.05)\) protein content when compared to that of the MOD and SEV fillets (Figure 4). Moreover, there was a difference \((P < 0.05)\) in the protein content in the dorsal and ventral surfaces of SEV fillets, even though a similar difference was not seen in NORM and MOD fillets. The relationship between the mean (average of the dorsal and ventral portions) protein and fat content of the fillets can be appreciated from Figure 5. There was large variation in the protein and fat contents in both MOD and SEV fillets which could have resulted in the significant negative correlation. The negative correlation implies that the protein in the muscle might be getting replaced by fat as suggested by the histopathological findings.

E. DISCUSSION

White striping has similarities to some of the muscle abnormalities already reported in chickens. The gross and/or the histopathology of the condition can be compared to hereditary muscular dystrophy (Julian and Asmundson, 1963; McMurtry et al., 1972; Julian, 1973), nutritional myopathy (Dam et al., 1952; Machlin and Shalkop, 1956; Klasing, 2008), deep pectoral myopathy (Wight and Siller, 1980) and toxic myopathy (Chalmers, 1981; Dowling, 1992; Roder, 2011). Moreover, the gross appearance of white striping meat and the increased
intramuscular fat seems to be similar to marbling in beef. The following discussion compares and contrasts white striping with the above conditions.

**Comparison of white striping with various muscle abnormalities in chickens**

Hereditary muscular dystrophy is a condition that is also characterized grossly by white striations of varying degree seen on pectoral muscle (Asmundson and Julian, 1956; Julian and Asmundson, 1963). Microscopic studies of the lesions revealed myopathic lesions similar to MOD and SEV degrees of white striping seen in the present study (Jordan et al., 1959; Holliday et al., 1968; McMurtry et al., 1972; Julian, 1973). The occurrence of the condition may result in increased fat content and change in the fatty acid profile of meat (Jordan et al., 1959; Jordan et al., 1964; Chio et al., 1972). Muscular dystrophy, however, is caused by a homozygous autosomal recessive gene (*am*), and the phenotypic expression depends on other modifying genes (Asmundson and Julian, 1956; Wilson et al., 1988). Furthermore, most of the studies mentioned above used specific dystrophic strains of chickens, while the reports on the occurrence in today’s commercial broiler strains are few. In contrast, Kuttappan et al. (2009) studied the incidence of white striping in four different commercial strains and reported the occurrence of higher degrees of white striping in all strains. In addition, there are a number of studies which estimated the occurrence of different degrees of white striping in various strains of broiler birds (Bauermeister et al., 2009; Kuttappan et al., 2012a; Kuttappan et al., 2012b). Though, some of the strains showed higher percentages of MOD and SEV, this could be associated with the higher body weight or enhanced growth rate (Kuttappan et al., 2009). These reports make it difficult to link hereditary muscle dystrophy and white striping. Nonetheless, it does not rule out the possibility of any underlying genetic defect associated with fast growing birds.
Nutritional myopathy is another condition that could be compared to white striping. Nutritional myopathy is associated with deficiency of vitamin E along with selenium and sulfur containing amino acids in poultry diets, and has gross and microscopic lesions similar to white striping (Dam et al., 1952; Machlin and Shalkop, 1956; Klasing, 2008). According to the National Research Council (1994), the required amount of vitamin E in broiler diet is 10 IU/kg of feed. The studies that reported the occurrence of MOD and SEV degrees of white striping, however, used poultry ration with adequate or higher levels of vitamin E and associated nutrients. Also, it is to be noted that the occurrence of NORM, MOD and SEV degrees was present in the same flock fed with the same diet treatment. More importantly, the study conducted by Kuttappan et al. (2012c) showed that even 400 IU of vitamin E/kg of feed could not completely prevent the occurrence of MOD and SEV white striping. This makes white striping less likely to be a vitamin E deficiency.

Deep pectoral myopathy is caused by the strangulation of supracoracoideus (or pectoralis minor) muscle due to lack of enough space between the tough inelastic fascia and keel bone leading to ischemia. It results in the necrosis of the muscle fibers in tenders resulting in greenish discoloration (Siller, 1985; Mitchell, 1999; Bilgili and Hess, 2002). The condition is mainly confined to tenders and the characteristic green appearance of the muscle on gross examination clearly suggests that it is different from white striping. However, deep pectoral myopathy has some similarities in the microscopic lesions seen in the supracoracoideus muscle (tenders) with that of white striping (Wight and Siller, 1980), which suggests that ischemia may also be associated with white striping.

There are reports of toxic myopathies in poultry which has microscopic lesions similar to white striping. The occurrence of toxic myopathies are associated with a number of feed
ingredients such as ionophore anticoccidial drugs (Dowling, 1992; Mitchell, 1999; Roder, 2011), *Cassia occidentalis* (Graziano et al., 1983; Mahon, 1999), gossypol in cotton seed meal, *Taxus spp.*, *Nerium oleander, Eupatorium rugosum, Karwinskia humboldtiana*, and vetch (Roder, 2011). In most of the studies which so far have reported the occurrence of severe degrees of white striping none of these plants were used as a feed ingredient (Kuttappan et al., 2009; Kuttappan et al., 2012b; Kuttappan et al., 2012c). The anticoccidial ionophore drug monensin was used in the above studies at levels of 90g/ton of feed which is within the therapeutic dose of the drug (Wagner et al., 1983). The lack of the typical clinical signs of ionophore toxicity like feed refusal, growth depression and creamy-colored diarrhea (Wagner et al., 1983; VanderKop et al., 1989) in white striping birds implies that the chance of toxic myopathic etiology is unlikely.

**Marbling and white striping**

Marbling refers to the white flecks of intramuscular fat deposit seen in meat. It is influenced by many factors like species, breed, gender, age, growth rate, muscle location and level of nutrition (Hocquette et al., 2010). The occurrence of marbling may enhance the flavor and juiciness of meat so it is considered as a superior quality in grading of beef, veal, mutton and lamb (USDA-FSIS, 2008). In poultry, majority of the fat is deposited as subcutaneous or abdominal fat while a little is being stored in muscle (Sams and Alvarado, 2010). Due to this reason, poultry meat is considered leaner leading to an increased preference among consumers (Davis and Stewart, 2002). However, the increased intramuscular fat deposit associated with MOD and SEV degree of white striping could give the appearance of marbling in chicken (Kuttappan et al., 2012a). An increase in intramuscular fat in marbling is mainly associated with increased deposition of fat in the perimysial layer (Moody and Cassens, 1968; Judge et al., 1989; Nishimura et al., 1999). In white striping, the increased fat deposit apparently replaces damaged
muscle fibers, and is therefore related to chronic degenerative myopathy. An increased
deposition of intramuscular fat may also occur in red muscle because of the increased blood
supply (Hocquette et al., 2010). In contrast, the present study showed that the higher degree of
white striping is seen in fillets of white muscle (Smith and Fletcher, 1988) compared to the
gastrocnemius or red muscle (Julian and Asmundson, 1963). Furthermore, occurrence of
marbling is mainly influenced by the breed of the animal (Crouse et al., 1989; Albrecht et al.,
2006). Based on the information available so far, white striping does not show any breed or
strain predilection rather it is more associated with heavier birds in all strains (Kuttappan et al.,
2009). Moreover, the intramuscular fat deposition in beef is inversely related to the muscle mass
(Albertí et al., 2008; Hocquette et al., 2010) whereas birds with heavier fillets have a greater
probability to have either MOD or SEV degrees of white striping (Kuttappan et al., 2012b;
Kuttappan et al., 2012c). From these observations, white striping in broilers and marbling in red
meat animals are two different conditions. In fact, the exact mechanisms which control the
occurrence of both marbling and white striping are still unknown which makes it difficult to
equate the two.

**Speculated pathogenesis in white striping**

The present study indicates that the severe degrees of white striping are
histopathologically characterized by chronic myopathic lesions. According to Dubowitz (1985),
myopathic lesions are nonspecific which could be occurring due to a number of neuromuscular
disorders (Valentine, 2008). Therefore, the precise etiology of white striping cannot be
confirmed. However, the possible etiology of white striping can be speculated based on the
information available so far. The comparison of white striping with existing muscular
abnormalities in chickens suggests that it could be an emerging condition which is closely
associated with the increase growth rate in broilers (Bauermeister et al., 2009; Kuttappan et al., 2009; Kuttappan et al., 2012b). Wilson et al. (1990) reported that the rapid growth rate in turkeys may have caused the muscle tissues to outgrow the limit of the supporting systems leading to a condition called focal myopathy, which is different from the deep pectoral myopathy or inherited muscular dystrophy. There are reports of muscular damage in turkeys which may be due to ischemia associated with rapid growth rate (Sosnicki et al., 1989; Sosnicki et al., 1991).

Furthermore, Mahon (1999) opined that intense selection for rapid growth rate in birds could have accidentally been accompanied by the selection for inadequate capillary/fascial growth or muscle fiber defects leading to myopathic changes referred to as growth-induced myopathy.

Also, there is a chance of reduced oxygen supply to breast muscle as a result of lower capillary density in fast growing chickens (Hoving-Bolink et al., 2000). The increased levels of serum enzymes indicating muscle damage along with myopathic changes was observed in birds with higher body weight (Macrae et al., 2006). The damage associated with higher growth rate can also lead to defective cation regulation resulting in increased level of sodium, potassium, magnesium and calcium in muscle tissue (Sandercock et al., 2009). An increased level of calcium in muscle tissue can initiate a number of tissue changes including the activation of intracellular proteases or lipases resulting in myopathic changes (Jackson et al., 1984; Mahon, 1999; Sandercock and Mitchell, 2003; Mitchell and Sandercock, 2004; Millay et al., 2009).

Furthermore, the inflammatory response during degeneration of myofibers will attract neutrophils, activated macrophages and T-lymphocytes to the area eventually resulting in phagocytosis of cell debris and release of factors such as cytokines, prostaglandins, etc. (Prisk and Huard, 2003; Smith et al., 2008). Also, the factors released from the inflammatory cells will activate of satellite cells initiating the regeneration of damaged myofibers (Kääriäinen et al.,
When the insult causing the muscle damage is “too great” or “too acute” or “too continuous”, the regenerative process will be ineffective (Mahon, 1999), eventually leading to fatty degeneration (Natarajan et al., 2010). In that case, the pluripotent stem cells in the muscle tissue differentiate to fibroblasts or adipocytes (Asakura et al., 2001; Wada et al., 2002; Shefer et al., 2004; Brack et al., 2007), due to the influence of the degenerating muscle fiber (Hosoyama et al., 2009), which ultimately results in fibrosis and lipidosis in the tissue.

In case of white striping, the enhanced growth rate associated with severe degrees could have resulted in over-stretching or ischemia in tissues resulting in muscle damage and initiation of reparative responses. Later, these reparative or regenerative attempts failed and took a different phase leading to fatty degeneration. This could be the reason why the MOD and SEV fillets have a higher lipidosis and fibrosis compared to the NORM. The presence of the acute and chronic myopathic lesions in the same tissue confirms the chronic ongoing insult which may have resulted in ineffective regenerative process. It should be noted that the gross lesions are more pronounced towards the cranial region of the ventral surface of the fillets where the fillets show maximum thickness. Furthermore, the myopathic lesions were higher in the ventral portion of the fillets where the convexity or stretching is greater when compared to the dorsal portion. Interestingly, there were differences in the occurrence of white striping in different muscles (Table 1). This implies that the development of lesions may be related to difference in the distribution of muscle fiber types. For example, it has been reported that white muscle fibers are predominately seen in pectoralis major (Smith and Fletcher, 1988) and iliotibialis muscle (Brackenbury and Williamson, 1989), while the gastrocnemius is primary composed of red muscle fibers (Julian and Asmundson, 1963). The present study showed a major difference in the occurrence of gross and microscopic white striping lesions in iliotibialis and pectoralis major.
(mainly towards the ventral portion) suggesting that the white fibers may be more susceptible to the condition. The selection of enhanced growth rate could have adversely affected the capillary supply to musculature leading to an increased percentage of white or glycolytic fibers in various skeletal muscles (Soike and Bergmann, 1998a). Furthermore, these glycolytic muscle fibers in fast growing birds may have reduced calcium transport abilities which make them vulnerable to pathologic changes due to the increased energy demand and production of lactate (Soike and Bergmann, 1998b). In addition, the difference in the growth rate for various muscle fiber types (Ono et al., 1993) may also be contributing to differences in the myopathic changes in these muscles. Nonetheless, the tremendous growth rate in modern broilers could result in greater incidence of higher degrees of white striping in the industry, leading to economic loss due to the rejection of poultry meat by the consumers (Kuttappan et al., 2012a). So, further studies are required to confirm the etiology and the possible ways to reduce the incidence of the condition in poultry meat.

To conclude, the occurrence of MOD and SEV degrees of white striping is histologically characterized by chronic myopathic lesions like loss of cross striations, variability in fiber size, floccular/vacuolar degeneration and lysis of fibers, mild mineralization, occasional regeneration (nuclear rowing and multinucleated cells), mononuclear cell infiltration, lipidosis and interstitial inflammation and fibrosis. The presence of polyphasic lesions on the same muscle sample indicates the chronic and ongoing insult causing these lesions. The increased incidence of these lesions is associated with changes in the proximate composition of meat, especially decrease in protein and increase in fat percentages. The occurrence of tissue changes are more frequent towards the cranio-ventral surface of the fillet compared to the other regions. Furthermore, lesions are more apparent in fillets and iliotibialis muscle compared to the tenders and
gastrocnemius muscles. Based on the tissues changes and the observations from previous studies, white striping could be an emerging issue in poultry meat industry and appears to be associated with enhanced growth rate in birds.
F. REFERENCES


Table 1: Comparison of the histopathological score (0/normal to 3/severe) for myopathic lesions (H & E staining) associated with white striping in different muscles

<table>
<thead>
<tr>
<th>Category²</th>
<th>Histopathological Score¹</th>
<th>Fillet (ventral)</th>
<th>Fillet (dorsal)</th>
<th>Tenders</th>
<th>Thigh</th>
<th>Drumstick</th>
</tr>
</thead>
<tbody>
<tr>
<td>NORM (n=6)</td>
<td>0.83ᵇ</td>
<td>0.08ᵇ</td>
<td>0.40ᵃ</td>
<td>0.33ˢ</td>
<td>0.92ᵃ</td>
<td></td>
</tr>
<tr>
<td>MOD (n=6)</td>
<td>2.33ᵃ</td>
<td>1.67ᵃ</td>
<td>1.20ᵃ</td>
<td>1.00ᵇ</td>
<td>1.08ᵃ</td>
<td></td>
</tr>
<tr>
<td>SEV (n=6)</td>
<td>2.83ᵃ</td>
<td>1.67ᵃ</td>
<td>1.42ᵃ</td>
<td>1.75ᵃ</td>
<td>1.17ᵃ</td>
<td></td>
</tr>
<tr>
<td>Pooled SE</td>
<td>0.18</td>
<td>0.24</td>
<td>0.38</td>
<td>0.17</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

ᵃᵇᶜ significant (P < 0.05) difference within each column

¹Scale used to assess myopathic lesions (H & E staining): 0=normal, 3=severe

²NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Figure 1: Micrographs of normal (a,c,e) and severe (b,d,f) samples in the histopathological scale (ranging 0 to 3) used to score the myopathic lesions (H & E; a,b), fibrosis (Masson’s Trichrome; c,d) and lipidosis (Oil red O ; e,f)
Figure 2: Histopathological scores for myopathic lesions (H & E), fibrosis and lipidosis in fillets from different degrees of white striping\(^1\) (n=20/each degree of white striping)

\(^{a-c}\) comparison between each degree of white striping within each histopathological characteristic

\(^1\)NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Figure 3: Fat content in breast fillets with different degrees of white striping\(^1\) (n=15/each degree of white striping)

\[^{a-c}\] comparison between each degree of white striping within ventral or dorsal portion
*significant \((P < 0.05)\) difference between the ventral or dorsal portion within MOD and SEV fillets
\(^1\)NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Figure 4: Protein content in breast fillets with different degrees of white striping.

*significant ($P < 0.05$) difference between the ventral/dorsal portion of SEV fillets

NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
Figure 5: Correlation between the mean (average of dorsal and ventral portions of the fillet) fat and protein in breast fillets with NORM ( ), MOD ( ) and SEV ( ) degrees of white striping

\[ y = -0.6568x + 95.652 \]
\[ R^2 = 0.1375 \]

\[ y = -1.0348x + 95.707 \]
\[ R^2 = 0.8479 \]

\[ y = -1.0958x + 97.826 \]
\[ R^2 = 0.8036 \]

1NORM = normal (no white striping); MOD = moderate degree; SEV = severe degree
G. APPENDIX

**Site of collection of muscle samples chicken carcass**

a. Tender (Pectoralis minor)
b. Fillet (Pectoralis major) – dorsal side*
c. Thigh (Iliotibialis)
d. Leg (Gastrocnemius)

*Ventral side of the fillet is the skin side which can be seen in this picture
VIII. COMPARISON OF HEMATOLOGIC AND SEROLOGIC PROFILES OF BROILER BIRDS WITH NORMAL (NORM) AND SEVERE (SEV) DEGREES OF WHITE STRIPING IN BREAST FILLETS

A. ABSTRACT

White striping is the white striation occasionally observed parallel to the direction of muscle fibers in broiler breast fillets and thighs at the processing plant. Broiler breast fillets can be categorized as normal (NORM), moderate (MOD), and severe (SEV) based on the degree of white striping. Histologically, the SEV fillets are characterized by the highest degree of degeneration of muscle fibers along with fibrosis and lipidosis when compared to NORM. The present study was undertaken to compare the hematologic and serologic profiles of broilers with NORM and SEV degrees of white striping in order to get more information on the systemic changes associated with the condition. In this study, day-old male broiler chicks of a commercial strain were grown on the same diet in 6 replicate pens (n = 32 birds/pen). Blood samples (5ml) were collected from the wing vein of each bird on the day before processing for analyzing hematologic and serologic profiles. At 63 d, the birds were weighed and processed in a commercial inline processing system. Weight of the butterfly fillets, liver, and abdominal fat pad were recorded. Left-side fillets were scored to obtain the degree of white striping for each bird. Representative samples for NORM (n = 24) and SEV (n = 17) categories were selected to compare the hematologic and serologic profiles. The SEV birds had greater ($P < 0.05$) live, fillet, and liver weights, as well as fillet yield, when compared to the NORM birds, but the abdominal fat yield was less ($P < 0.05$) in SEV birds. The NORM and SEV birds did not show any differences in various hematological parameters, including the differential leucocyte count. Conversely, the SEV birds had elevated ($P < 0.05$) serum levels of creatine kinase, alanine...
transaminase, aspartate aminotransferase, and lactate dehydrogenase. These results suggest that there is no systemic infectious or inflammatory condition associated with SEV degree of white striping. The elevated serum enzyme levels confirm the muscle damage associated with the degenerative myopathy in SEV birds.

B. INTRODUCTION

White striations occurring parallel to the direction of muscle fibers in broiler breast fillets, referred to as white striping, are causing concern among broiler chicken producers due to reduced consumer appeal. Depending on the severity of the condition, broiler breast fillets can be categorized as no striping or normal (NORM), moderate (MOD) and severe (SEV) (Kuttappan et al., 2012a). NORM fillets do not show any distinct white lines while there will be white lines of < 1mm and > 1mm thick in MOD and SEV fillets, respectively. Furthermore, the higher degrees of white striping are associated with heavier birds (Bauermeister et al., 2009; Kuttappan et al., 2009) or birds with increased growth rate (Kuttappan et al., 2012b). This suggests that the increased growth rate of poultry, accompanied with the selection for greater growth rates and grow-outs of broilers in a short period of time could produce a greater incidence of the condition in the meat market. Recently, Kuttappan et al. (2012a) reported that visual acceptance of broiler breast fillets can be significantly reduced due to the occurrence of MOD and SEV degree of white striping. Because visual appearance is a major attribute contributing to the purchase of raw breast meat, the higher incidence of white striping could result in economic loss for the producer. Though the condition is present prior to processing, it is only visible after processing when breast meat is exposed. So far, few studies are being conducted on systemic changes occurring in live birds with this condition. The knowledge about the systemic changes may help us to get valuable information about the etiology of tissue changes associated with the condition.
Kuttappan et al. (2011) evaluated the histology of the condition and reported that the higher degrees of white striping are associated with damage of muscle fibers. According to Valentine and McGavin (2012), one of the reasons for the muscle damage could be an infectious or inflammatory condition associated with a disease condition affecting the muscle tissue. Because white striping is a newly reported condition, there are no reports of any associated pathogen so far. However, this possibility should not be overlooked. Hematology is the common clinical tool used for the diagnosis of various disease conditions. There could be differences in the peripheral blood profile, mainly in total leucocyte count and leucocyte differential count, during conditions like infection (bacterial, viral, or fungal), inflammation, or stress (Mitchell and Johns, 2008; Doneley and Doneley, 2010; Huff et al., 2010). In chickens, an inflammatory or infectious condition could cause a pronounced increase in heterophil count, which could even result in the reversal of the normal lymphocyte dominance (Latimer and Bienzle, 2010). Also, the damage occurring in the muscle tissue could be reflected in plasma or serum biochemical profiles. The condition could disrupt the integrity of the sarcolemma resulting in the leaking of various enzymes such as creatine kinase (CK), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) into the plasma or serum (Hochleithner, 1994; Hoffman and Solter, 2008).

In addition, white striping is histologically characterized with localized lipidosis associated with necrotic myofibers (Kuttappan et al., 2011), and proximate analysis revealed a greater amount of fat with differences in the fatty acid profile of SEV samples when compare to NORM (Kuttappan et al., 2012b). Avian adipocytes contribute little to de novo lipogenesis (Griffin et al., 1992). Therefore, increased fat deposition in birds could be due to either increased synthesis and secretion from liver or increased break down, uptake and storage in adipocytes.
The increased lipogenesis in chickens could be associated with a greater ratio of liver to live weight and differences in fatty acid profiles (Saadoun and Leclercq, 1987). Furthermore, the increased lipogenesis in liver could be associated with enhanced secretion of very low density lipoprotein (VLDL) which increases the amount of circulating triglycerides in birds (Hermier, 1997). Therefore, a detailed study on liver fatty acid profile and the serum biochemistry will aid in understanding the source of increased intramuscular fat in SEV birds. Based on all the above observations, it can be hypothesized that the occurrence of white striping could be associated with systemic changes which are manifested as variations in blood profile. A comparison of the liver and breast meat fatty acid profiles could provide supporting evidence for the above systemic changes. Thus, the objective of the study was to compare the hematologic and serologic indices as well as the liver and breast muscle fatty acid profiles of birds with NORM and SEV degree of white striping.

C. MATERIALS AND METHODS

Management of birds

All the procedures involving the birds used in the present experiment were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC protocol # 09035). In this study, day-old male chicks (n = 192) of a commercial strain were grown on the same diet treatment, which met or exceeded the NRC recommendations (National Research Council, 1994). There were 6 replicate pens (32 birds/pen), and the birds were provided with starter (0 to 14 d), grower (14 to 28 d) and finisher (28 to 63 d) diets. The birds had ad libitum access to feed and water throughout the period of study. At 63 d, 25 birds were randomly selected from each pen, weighed, and processed under standard conditions of University of
Arkansas Poultry Processing Pilot Plant (Mehaffey et al., 2006). Butterfly fillet, liver, and abdominal fat pad weights were recorded. Left-side fillet from each bird was visually scored to determine the degree of white striping (Kuttappan et al., 2012a). Briefly, fillets with apparently no white striation were considered as no striping, or NORM samples, whereas those with striations (mostly >1mm thick) as SEV. Meat (from the ventral or skin side region of the cranial part of left side fillets) and liver samples were collected from the birds with NORM (n = 5) and SEV (n = 5) degrees of white striping for fatty acid analysis.

**Blood and serum analysis**

Non-fasting blood samples (5 ml) were collected from the birds by venipuncture (wing vein) on the morning of 62 d (day before processing). The samples were collected in glass tubes without and with anticoagulant (EDTA-coated tubes). The hematologic profile of the whole blood samples were estimated using the Cell-Dyn 3500 blood analysis system (Abbott Diagnostics, Abbott Park, IL) which was standardized for chicken blood. The analysis uses electronic impedance and laser-light scattering to estimate total red blood cell (RBC) count, hemoglobin concentration (HGB), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and red cell distribution width (RDW), total white blood cell (WBC) or leucocyte count, and the percentage of heterophils (HET), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS) and basophils (BASO). Hematologic parameters for all the samples were estimated within 4 h of collection. Blood collected for serological tests was held at room temperature for 2 to 3 h, centrifuged (2,000 g for 10 min), and the resultant serum was stored at -20°C until analyzed. Later, all the serum samples were thawed and clinical chemistry was analyzed at the same time. An Express Plus automated clinical chemistry analyzer (Ciba-Corning Diagnostics Corp.,
Medfield, MA) was used to estimate the serum enzyme activity of alanine aminotransferase (ALT), alkaline phosphatase (AP), aspartate aminotransferase (AST), creatine kinase (CK), \( \gamma \)-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and levels of total protein (TP), albumin, glucose, triglycerides (TG), cholesterol, uric acid (UA), blood urea nitrogen (BUN), phosphorus, calcium, and iron. The system was initially standardized for poultry serum.

**Fatty acid analysis**

Meat and liver samples (n=5 each/ NORM or SEV) were randomly selected for fatty acid analysis. The samples were freeze dried and pulverized prior to the analysis. Fatty acid profile was estimated with the procedure described by Apple et al. (2009), with modifications as explained by Kuttappan et al. (2012b) in Chapter V. Total fatty acid and individual fatty acid methyl esters (FAME) contents were reported in units of mg of FAME/g of dry sample and FAME weight percentages, respectively.

**Statistical analysis**

The present study used birds from the same strain, gender, age, and dietary treatment in order to avoid any confounding effect from these factors. Each bird was considered as an individual experimental unit. All the data were analyzed using the GLM procedure of SAS (SAS Institute Inc.) with the degree of white striping as the main effect. The least square means were separated using Student’s t test or Tukey’s HSD test at a significance of \( P < 0.05 \).

**D. RESULTS AND DISCUSSION**

The incidence of NORM, MOD and SEV degrees of white striping in the present study were 16.67, 71.53 and 11.81%, respectively. Birds showing only NORM (n = 24) and SEV (n =
degree of white striping were considered for the comparative study. The SEV birds had heavier \((P < 0.05)\) live and fillet weights and greater \((P < 0.05)\) fillet yield when compared to NORM (Table 1). This was in accordance with the findings from a number of previous studies which reported that the higher degrees of white striping were associated with heavier birds (Bauermeister et al., 2009; Kuttappan et al., 2009; 2012b). In addition, the SEV birds had greater \((P < 0.05)\) liver weight but no difference \((P > 0.05)\) in liver yield (Table 1). Presumably, the increased liver weight in SEV birds is proportion to the increased size, or live weight, of the bird which was not observed in liver yield. The NORM birds had greater \((P < 0.05)\) abdominal fat yields than SEV birds, but there was no difference in the weight of abdominal fat pad between the 2 groups. A negative genetic correlation has been reported between abdominal fat and body weight (Leenstra et al., 1986) or breast meat yield (Cahaner et al., 1986; Le Bihan-Duval et al., 1998; Zerehdaran et al., 2004). This could be the reason why the SEV birds, which had greater \((P < 0.05)\) live weights and fillet yields, had a lower \((P < 0.05)\) abdominal fat yield (Table 1).

Blood from NORM and SEV birds did not reveal any differences \((P > 0.05)\) in total RBC, WBC, HCT, HGB, MCV, MCH, MCHC, RDW, and percentages of HET, LYM, MONO, EOS, and BASO (Table 2). In general, an increase in WBC count in the peripheral blood is often observed in stress, inflammatory condition due to generalized or localized infections, trauma, toxicities, neoplasms etc., whereas a decrease could be an indication of chronic inflammation or infections (Campbell, 1994; Doneley and Doneley, 2010). However, changes in specific cell population may be seen in various conditions. An increase in HET will be observed in bacteria, fungal and parasitic infections, inflammation, stress, toxicities, traumatic conditions and leukemia (Campbell, 1994; Mitchell and Johns, 2008). Certain infectious conditions, like overwhelming bacterial infection or viral diseases of hemapoiteic cells, could cause a reduction
in HET count (Latimer and Bienzle, 2010). Increase in LYM will be mainly associated with antigens stimulation from chronic infections or inflammatory conditions which could be associated with certain viral infections (Mitchell and Johns, 2008; Doneley and Doneley, 2010), and a reduction in LYM in case of stress conditions (Huff et al., 2010; Latimer and Bienzle, 2010). Depending upon various conditions, a shift in the number of HET or LYM can result in a change in the HET: LYM ratio as well. Increased number of MONO, BASO and EOS can also be seen in association with various infectious or inflammatory conditions (Latimer and Bienzle, 2010). Meanwhile, there could be a decrease in the HCT, HGB, MCV, and MCH levels in birds when they are exposed to stressors (Borges et al., 2004; Huff et al., 2008). The RBC count, hematocrit, hemoglobin, MCV, MCHC, and RDW are also used to determine the presence and severity of anemia (Tvedten, 2010). Based on the available literature, the lack of any differences in various hematologic parameters in the present study indicate that the occurrence of SEV degrees of white striping is not associated with infection, inflammation, or stress which could produce generalized systemic changes.

Serum enzyme levels were estimated in order to assess tissue damages associated with white striping. There was an increase (\( P < 0.05 \)) in the serum levels of ALT, AST, CK, and LDH in SEV birds when compared to the NORM (Table 3). Increased levels of ALT, AST, and LDH levels are associated with liver or muscle damage (Hochleithner, 1994; Lumeij, 2008). Creatine kinase is an enzyme which is more specific for skeletal muscle and is often used to distinguish whether increased concentrations of AST and ALT are from either liver or muscle damage (Hoffman and Solter, 2008). Furthermore, the half-life of the above enzymes are in the increasing order LDH < CK < AST < ALT (Lumeij, 2008), and this differences in half-life is an effective tool for determining the acute or chronic nature of the condition. Meanwhile, GGT is an
indicator of hepatocellular and renal damage, which is manifested as increased levels in serum or plasma and urine, respectively (Hochleithner, 1994; Hoffman and Solter, 2008). Moreover, there was no difference ($P > 0.05$) in serum GGT levels in NORM and SEV birds (Table 3). These data suggest that the occurrence of SEV degree of white striping is associated with muscle damage and not with liver abnormalities. In addition, the increase in serum AST and ALT indicates the chronicity of muscle damage while the simultaneous increase in LDH and CK concentrations indicate that it is ongoing. This is consistent with the chronic degeneration of muscle fibers observed in histological samples prepared from breast fillets of SEV birds also having greater body weight compared to NORM (Kuttappan et al., 2009; 2011). Similarly, the incidence of increased muscle damage was reported in fast growing turkeys as well (Sosnicki et al., 1989; Sosnicki et al., 1991). Interestingly, the SEV birds showed lower ($P < 0.05$) serum levels of AP when compared to NORM (Table 3). Higher serum levels of AP are observed when there is increased osteoblastic activity, involving formation and mineralization of bone associated with increased skeletal growth (Lumeij, 2008). Szabo et al. (2005) reported that reduced activity of AP may be an indication of slowdown of bone growth observed when the birds reach the adult body mass. Perhaps, the SEV birds, with greater body weight, may be more near to the adult body mass when compared to NORM resulting in reduced bone growth and serum AP levels. In addition to similar GGT serum levels, there were no differences in the serum levels of cholesterol and TG, which again suggests similar hepatic activity in the 2 groups (Hochleithner, 1994; Hermier, 1997). There were no differences in the levels of BUN, creatinine, phosphorus, and UA (Table 3), suggesting the lack of differences in renal function between NORM and SEV birds (Hochleithner, 1994). Similarly, the 2 groups did not show any disparity in serum levels of albumin, calcium, glucose, TP, magnesium, and iron (Table 3) indicating on
difference in the absorption or metabolism of these substances. The differences in serum enzyme levels between the NORM and SEV birds indicate muscle damage in SEV birds. Nonetheless, a similar pattern of enzyme levels can be seen in various conditions like stress, increased muscle growth or activity, intramuscular injections, injuries (such as broken wings), or other disease conditions causing muscle damage (Mills et al., 1998; Szabo et al., 2005; Macrae et al., 2006; Hoffman and Solter, 2008; Huff et al., 2008; Huff et al., 2010). So, further comparative studies involving the type and the extent in elevation of various enzyme levels are needed in order to confirm the potential of the tool to distinguish white striping from other similar conditions.

Fatty acid content of liver and breast muscle sample were determined to compare it with the serum biochemistry and hypothesize the reasons for the changes associated with white striping. The SEV samples showed a higher ($P < 0.05$) amount of total fatty acids (Figure 1) in breast meat when compared to NORM. The SEV breast fillets had a lower ($P < 0.05$) proportion of saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA), but a greater ($P < 0.05$) percentage of monounsaturated fatty acid (MUFA), than the NORM breast fillets (Table 4). Among the 2 white striping categories, the SEV breast fillets had greater ($P < 0.05$) amounts of the SFAs such as lauric, myristic, pentadecanoic, heptadecanoic, and arachidic acids, whereas NORM fillets had a greater ($P < 0.05$) percentage of stearic acid. There were no differences ($P > 0.05$) in palmitic acid percentages, but SEV breast fillets had greater ($P < 0.05$) proportions of the MUFAs such as myristoleic, palmitoleic, palmitelaidic, and oleic acids, whereas NORM fillets had a greater ($P < 0.05$) percentages of vaccenic acid. The levels of linoleic, as well as $\alpha$- and $\gamma$-linolenic, acids were elevated in SEV breast fillets, and greater weight percentages of arachidonic acid, docosapentaenoic (DPA), docosahexanoic (DHA), eicosadienoic, and dihomo-$\gamma$-linolenic acids were observed in NORM fillets. Interestingly, the magnitude of difference in
the total fatty acid content in NORM and SEV breast fillets observed in the present study was
greater than 3 times of that reported by Kuttappan et al. (2012b) although, the overall trend seen
in the fatty acid profiles of NORM and SEV breast fillets was quite similar. However, there were
differences with respect to the absolute values of individual fatty acids which could be due to the
difference in the strain, age and feed used between the 2 studies (Wood and Enser, 1997;
Nürnberg et al., 1998; Poureslami et al., 2010).

The total fatty acid content in liver and breast meat was not different \((P > 0.05)\) in SEV
birds, but NORM breast meat had less \((P < 0.05)\) total fatty acid content than that of the NORM
liver (Figure 1). In addition, total fatty acids, SFA, MUFA, and PUFA contents of liver samples
from both NORM and SEV did not differ \((P > 0.05)\) between fillets. Nevertheless, the levels of
palmitic acid and DHA were greater \((P < 0.05)\) in the liver of NORM and SEV, respectively
(Table 4). According to Bruss (1997), the major fatty acids synthesized in animal tissue result
from \textit{de novo} lipogenesis of palmitic and stearic acids. The increased \((P < 0.05)\) level of palmitic
acid may be an indication of increased lipogenesis in the liver of NORM birds compared to the
SEV. However, such a difference was not seen in the levels of palmitic and stearic acid in the
breast muscle and stearic acid level of liver from both NORM and SEV birds. In case of animals,
diet is the major source of linoleic and \(\alpha\)-linolenic acids because they are not synthesized in the
animal body (Cook, 1996) and the present study used the same diet formulation for all the birds.
In this case, the increased levels of linoleic and \(\alpha\)-linolenic acids in breast muscle of SEV birds
imply an increased uptake of dietary fat into the adipocytes in breast muscle. For that to happen,
there should be higher lipoprotein lipase activity, plausibly from adipocyte hyperplasia, in these
tissues which will help the breakdown and uptake of circulating triglycerides (Hermier et al.,
1989). Recently, it has been reported that degeneration of muscle fibers from irreparable muscle
damage could result in the differentiation of muscle stem cells to adipocytes or fibroblasts (Hosoyama et al., 2009; Joe et al., 2010; Natarajan et al., 2010). Histopathological studies reported that the SEV degree of white striping could be associated with degeneration of muscle fibers along with lipidosis and fibrosis (Kuttappan et al., 2009; 2011). The degenerative muscle fibers may have resulted in hyperplasia of adipocytes in SEV breast fillets leading to increased uptake of circulating triglycerides and storage of intramuscular fat compared to NORM breast muscle. It has been observed that higher degrees of white striping are closely related to heavier birds (Bauermeister et al., 2009; Kuttappan et al., 2009) or birds with higher growth rates (Kuttappan et al., 2012b). Various studies have already shown that the rapid and enhanced growth in birds could be associated with muscle damages because of inability of the supporting system to cope up with the increasing demand (Wilson et al., 1990; Mills et al., 1998; Macrae et al., 2006). This implies that increased growth associated with SEV white striping birds could have caused the degenerative changes in muscle fibers eventually resulting in a higher amount of intramuscular fat deposition. Meanwhile, it is also possible that the availability of space due to the degeneration of muscle fibers may have resulted in expansion of adipocytes which makes it capable of storing more fat (Kuttappan et al., 2012b). Furthermore, α-linolenic acid is the precursor for EPA and DHA (Wood and Enser, 1997), therefore, the SEV fillets are expected to have a higher amount of EPA and DHA based on the high α-linolenic acid content. However, such a relation was not observed in the present study, which is in agreement with the findings of Kuttappan et al. (2012b). Previously, some of the studies reported the same outcome which could be due to the accumulation of synthesized EPA and DHA in liver and other tissues (Ajuyah et al., 1993; Rymer and Givens, 2005). This may be the reason why the liver from SEV birds had a greater amount of DHA when compared to that of NORM birds.
In conclusion, SEV degree of white striping is related to heavier birds as already reported by previous studies. The occurrence of the condition could be associated with decreased abdominal fat yield, but not liver yield. The hematologic profile suggested that SEV white striping was not associated with any infectious, inflammatory or stress condition. As a result of the muscle damage in SEV breast fillets, there was an increase in serum levels of CK, ALT, AST, and LDH. Birds with SEV degree of white striping may have increased intramuscular fat deposition, and the fat deposited has a different fatty acid profile when compared to NORM. However, there is no difference in liver fat content between the NORM and SEV birds. Overall, liver yield (as a percentage of live weight), serologic profile, total fatty acid content and the fatty acid profiles of liver and breast meat from NORM and SEV birds suggest that the increased intramuscular fat deposited in SEV breast fillet could not be a result of increased de novo lipogenesis. Presumably, an adipocytic hyperplasia, originating from the ongoing muscle damage, has resulted in enhanced break down, uptake and storage of circulating TG in SEV breast fillets.
E. REFERENCES


Table 1: Comparison of carcass yield (mean ± SE) from NORM and SEV birds

<table>
<thead>
<tr>
<th>Attributes</th>
<th>NORM(^1) (n=24)</th>
<th>SEV(^2) (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live bird</td>
<td>4070.17(^b) ± 77.36</td>
<td>4736.88(^a) ± 91.91</td>
</tr>
<tr>
<td>Fillet</td>
<td>754.46(^b) ± 24.35</td>
<td>1076.71(^a) ± 28.93</td>
</tr>
<tr>
<td>Liver</td>
<td>52.46(^b) ± 1.64</td>
<td>59.00(^a) ± 2.01</td>
</tr>
<tr>
<td>Abdominal fat pad</td>
<td>50.04(^a) ± 3.32</td>
<td>46.35(^a) ± 3.94</td>
</tr>
<tr>
<td>Yield (% of live weight)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fillet</td>
<td>18.47(^b) ± 0.34</td>
<td>22.70(^a) ± 0.40</td>
</tr>
<tr>
<td>Liver</td>
<td>1.29(^a) ± 0.03</td>
<td>1.25(^a) ± 0.04</td>
</tr>
<tr>
<td>Abdominal fat pad</td>
<td>1.21(^a) ± 0.07</td>
<td>0.98(^b) ± 0.08</td>
</tr>
</tbody>
</table>

\(^{a-b}\) Means in each row with different letters are significantly different (\(P < 0.05\))

1 NORM – normal (no white striping)

2 SEV – severe degree of white striping
Table 2: Comparison of hematologic profiles (mean ± SE) of NORM and SEV birds

<table>
<thead>
<tr>
<th>Attributes</th>
<th>NORM&lt;sup&gt;1&lt;/sup&gt; (n=24)</th>
<th>SEV&lt;sup&gt;2&lt;/sup&gt; (n=17)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White blood cell (WBC) count (x 10&lt;sup&gt;3&lt;/sup&gt;/µL)</td>
<td>28.04 ± 2.44</td>
<td>24.28 ± 2.90</td>
</tr>
<tr>
<td>Heterophil, HET (%)</td>
<td>36.15 ± 2.79</td>
<td>36.20 ± 3.31</td>
</tr>
<tr>
<td>Lymphocytes, LYM (%)</td>
<td>52.50 ± 3.49</td>
<td>54.85 ± 4.14</td>
</tr>
<tr>
<td>HET:LYM</td>
<td>0.95 ± 0.13</td>
<td>0.75 ± 0.16</td>
</tr>
<tr>
<td>Monocytes, MONO (%)</td>
<td>10.51 ± 0.90</td>
<td>8.54 ± 1.07</td>
</tr>
<tr>
<td>Eosinophils, EOS (%)</td>
<td>0.03 ± 0.01</td>
<td>0.03 ± 0.01</td>
</tr>
<tr>
<td>Basophils, BASO (%)</td>
<td>0.81 ± 0.16</td>
<td>0.36 ± 0.19</td>
</tr>
<tr>
<td>Red blood cell (RBC) count (x 10&lt;sup&gt;6&lt;/sup&gt;/µL)</td>
<td>2.69 ± 0.04</td>
<td>2.67 ± 0.05</td>
</tr>
<tr>
<td>Hematocrit, HCT (%)</td>
<td>35.88 ± 0.51</td>
<td>35.82 ± 0.61</td>
</tr>
<tr>
<td>Hemoglobin, HGB (g/dL)</td>
<td>8.05 ± 0.09</td>
<td>8.16 ± 0.11</td>
</tr>
<tr>
<td>Mean cell volume, MCV (fL)</td>
<td>133.54 ± 0.74</td>
<td>133.41 ± 0.88</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin , MCH (pg)</td>
<td>30.00 ± 0.34</td>
<td>30.71 ± 0.40</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration, MCHC (g/dL)</td>
<td>24.55 ± 1.61</td>
<td>22.84 ± 1.91</td>
</tr>
<tr>
<td>Red cell distribution width, RDW (%)</td>
<td>10.99 ± 0.30</td>
<td>10.69 ± 0.36</td>
</tr>
</tbody>
</table>

Means in each row were not significantly different (P > 0.05)

<sup>1</sup> NORM – normal (no white striping)

<sup>2</sup> SEV – severe degree of white striping
Table 3: Serological profile of birds with NORM and SEV degree of white striping

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NORM(^1) (n=24)</th>
<th>SEV(^2) (n=17)</th>
<th>Pooled SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enzymes (U/L)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine Aminotransferase (ALT)</td>
<td>2.25</td>
<td>4.58</td>
<td>0.50</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Alkaline Phosphatase (AP)</td>
<td>2169.17</td>
<td>1469.41</td>
<td>193.24</td>
<td>0.01</td>
</tr>
<tr>
<td>Asparate Aminotransferase (AST)</td>
<td>607.75</td>
<td>1066.71</td>
<td>123.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Creatine Kinase (CK)</td>
<td>51350.00</td>
<td>123973.53</td>
<td>15707.03</td>
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<tr>
<td>Gamma Glutamyl Transferase (GGT)</td>
<td>25.67</td>
<td>25.65</td>
<td>2.12</td>
<td>0.99</td>
</tr>
<tr>
<td>Lactate Dehydrogenase (LDH)</td>
<td>3101.67</td>
<td>7024.71</td>
<td>1002.99</td>
<td>0.01</td>
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<tr>
<td><strong>Metabolites and electrolytes</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>1.45</td>
<td>1.44</td>
<td>0.03</td>
<td>0.76</td>
</tr>
<tr>
<td>Blood Urea Nitrogen, BUN (mg/dL)</td>
<td>0.43</td>
<td>0.41</td>
<td>0.08</td>
<td>0.82</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>10.05</td>
<td>10.02</td>
<td>0.17</td>
<td>0.92</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>117.46</td>
<td>118.18</td>
<td>4.14</td>
<td>0.90</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.20</td>
<td>0.22</td>
<td>0.01</td>
<td>0.16</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>205.17</td>
<td>203.24</td>
<td>2.41</td>
<td>0.54</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
<td>1.75</td>
<td>1.69</td>
<td>0.07</td>
<td>0.46</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>4.14</td>
<td>4.19</td>
<td>0.10</td>
<td>0.68</td>
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<tr>
<td>Iron (µg/dL)</td>
<td>200.58</td>
<td>199.18</td>
<td>7.62</td>
<td>0.89</td>
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<tr>
<td>Total protein, TP (g/dL)</td>
<td>3.34</td>
<td>3.27</td>
<td>0.09</td>
<td>0.56</td>
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<tr>
<td>Triglycerides, TG (mg/dL)</td>
<td>57.79</td>
<td>48.53</td>
<td>5.77</td>
<td>0.23</td>
</tr>
<tr>
<td>Uric acid, UA (mg/dL)</td>
<td>6.69</td>
<td>5.99</td>
<td>0.43</td>
<td>0.22</td>
</tr>
</tbody>
</table>

\(^1\) NORM – normal (no white striping)

\(^2\) SEV – severe degree of white striping
<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Liver (n=5)</th>
<th>SEV (n=5)</th>
<th>Pooled SE</th>
<th>P value</th>
<th>Liver (n=5)</th>
<th>SEV (n=5)</th>
<th>Pooled SE</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SFA</strong></td>
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</tr>
<tr>
<td>Lauric acid (12:0)</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Myristic acid (14:0)</td>
<td>0.29</td>
<td>0.26</td>
<td>0.02</td>
<td>0.23</td>
<td>0.55</td>
<td>0.66</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Palmitic acid (16:0)</td>
<td>20.18</td>
<td>18.91</td>
<td>0.21</td>
<td>&lt;0.01</td>
<td>23.34</td>
<td>22.5</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Heptadecanoic acid (17:0)</td>
<td>0.22</td>
<td>0.23</td>
<td>0.01</td>
<td>0.44</td>
<td>0.12</td>
<td>0.2</td>
<td>0.02</td>
<td>&lt;0.01</td>
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<tr>
<td>Stearic acid (18:0)</td>
<td>18.54</td>
<td>18.93</td>
<td>0.52</td>
<td>0.60</td>
<td>9.04</td>
<td>8.03</td>
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<td>&lt;0.01</td>
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<td><strong>MUFA</strong></td>
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<tr>
<td>Myristoleic acid (14:1)</td>
<td>0.04</td>
<td>0.02</td>
<td>0.01</td>
<td>0.28</td>
<td>0.01</td>
<td>0.14</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Palmitoleic acid (16:1c)</td>
<td>1.76</td>
<td>1.63</td>
<td>0.17</td>
<td>0.59</td>
<td>3.46</td>
<td>4.07</td>
<td>0.13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Palmitelaidic acid (16:1t)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.85</td>
<td>0</td>
<td>0.03</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Oleic acid (18:1c9)</td>
<td>16.98</td>
<td>16.3</td>
<td>0.87</td>
<td>0.59</td>
<td>26.55</td>
<td>29.9</td>
<td>0.45</td>
<td>&lt;0.01</td>
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<tr>
<td>Summation of unseparable 18:1t acids</td>
<td>0.3</td>
<td>0.3</td>
<td>0.01</td>
<td>0.82</td>
<td>0.25</td>
<td>0.37</td>
<td>0.02</td>
<td>&lt;0.01</td>
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<td><strong>PUFA</strong></td>
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<tr>
<td>Linoleic acid (18:2n-6)</td>
<td>19.38</td>
<td>19.97</td>
<td>0.32</td>
<td>0.20</td>
<td>21.63</td>
<td>22.86</td>
<td>0.35</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CLA 9-cis, 11-cis (18:2c9c11)</td>
<td>0.02</td>
<td>0</td>
<td>0.01</td>
<td>0.12</td>
<td>0</td>
<td>0.03</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CLA 9-cis, 11- trans (18:2c9t11)</td>
<td>0.01</td>
<td>0</td>
<td>&lt;0.01</td>
<td>0.15</td>
<td>0</td>
<td>0.03</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>α- Linolenic acid ME (18:3n-3)</td>
<td>0.37</td>
<td>0.41</td>
<td>0.02</td>
<td>0.20</td>
<td>0.64</td>
<td>0.87</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>γ- Linolenic acid ME (18:3n-6)</td>
<td>0.31</td>
<td>0.29</td>
<td>0.01</td>
<td>0.49</td>
<td>0.19</td>
<td>0.27</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>Eicosadienoic acid (20:2n-6)</td>
<td>0.44</td>
<td>0.45</td>
<td>0.02</td>
<td>0.85</td>
<td>0.45</td>
<td>0.34</td>
<td>0.02</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eicosatrienoic acid (20:3n-3)</td>
<td>0.05</td>
<td>0.06</td>
<td>&lt;0.01</td>
<td>0.09</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Dihomo- γ-linolenic acid (20:3n-6)</td>
<td>1.12</td>
<td>0.9</td>
<td>0.08</td>
<td>0.06</td>
<td>0.66</td>
<td>0.49</td>
<td>0.07</td>
<td>0.05</td>
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<tr>
<td>Arachidonic acid (20:4n-6)</td>
<td>11.97</td>
<td>13</td>
<td>0.49</td>
<td>0.15</td>
<td>5.77</td>
<td>3.11</td>
<td>0.31</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Eicosapentaenoic acid, EPA (20:5n-3)</td>
<td>0.29</td>
<td>0.26</td>
<td>0.02</td>
<td>0.29</td>
<td>0.08</td>
<td>0.09</td>
<td>0.02</td>
<td>0.86</td>
</tr>
<tr>
<td>Docosapentaenoic acid, DPA (22:5n3)</td>
<td>0.57</td>
<td>0.61</td>
<td>0.03</td>
<td>0.48</td>
<td>0.62</td>
<td>0.3</td>
<td>0.03</td>
<td>&lt;0.01</td>
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<tr>
<td>Docosahexaenoic acid, DHA (22:6n3)</td>
<td>0.92</td>
<td>1.10</td>
<td>0.05</td>
<td>0.01</td>
<td>0.41</td>
<td>0.17</td>
<td>0.03</td>
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<tr>
<td>Other fatty acid peaks</td>
<td>4.17</td>
<td>4.31</td>
<td>0.16</td>
<td>0.53</td>
<td>2.76</td>
<td>2.55</td>
<td>0.11</td>
<td></td>
</tr>
</tbody>
</table>

< 0.01
Figure 1: Fatty acid content in liver (n = 5) and breast (n = 5) muscle samples collected from NORM¹ and SEV² birds

¹ NORM – normal (no white striping)
² SEV – severe degree of white striping

a-b significant (P < 0.05) difference within and between the liver and breast samples from NORM and SEV birds
I. CONCLUSION

The results from this dissertation provide valuable information on the consumer acceptance and the characteristics of NORM (normal or no striping), MOD (moderate degrees) SEV (severe degrees) of white striping. Most of the consumers considered white striping as fatty or marbled appearance of broiler breast fillets resulting in reduced acceptance and purchase intent for with MOD and SEV degrees of white striping. This implies that the gross white lines on boneless skinless breast fillets could be a potential threat to the poultry meat market. However, the condition is less apparent after cooking and does not cause any solemn impact on cooked meat quality, especially tenderness. Elevated levels of serum enzymes like levels of creatine kinase, alanine transaminase, aspartate aminotransferase and lactate dehydrogenase in birds with SEV white striping birds, when compared to the NORM birds, indicated the possibility of increased muscle damage. The histopathology of MOD and SEV degree of white striping revealed muscle damage with chronic myopathic lesions like floccular/vacuolar degeneration and lysis of fibers, mononuclear cell infiltration, lipidosis and interstitial inflammation and fibrosis. Moreover, these tissue changes were reflected as differences in proximate composition of white striping meat, mainly a high fat and low protein contents. The lack of any strain predilection and effect of different levels of dietary vitamin E on the incidence suggest that the white striping may have a different etiology from similar and already known conditions like hereditary muscular dystrophy or nutritional myopathy, respectively. It has been strongly suggested that the higher degrees of white striping is associated with birds with higher live and RTC weights as well as heavier/thicker fillets. Furthermore, the high calories diets used to induce greater growth rate in broiler birds can result in increased incidence of white striping. In short, white striping could be a growth associated myopathy seen in broiler birds which could have serious impact on the
proximate composition and visual appearance of poultry meat. Further studies are warranted to estimate the incidence of white striping in the industry and also to unveil the cellular level changes which cause the condition.