INTRODUCTION

Visual appearance is the single meat quality that 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 other superior qualities. Recently, Kuttappan et al. (2012c) reported that white striping is a condition that could reduce the consumer acceptance and purchase intent for boneless skinless broiler breast fillets. The condition is characterized by white striation observed in the MOD and SEV samples consisted of loss of cross striations, variability in fiber size, flocular/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 for degeneration and necrosis, fibrosis, and lipidosis. The scale used to score the samples ranged from 0 (normal) to 3 (severe). There was an increase ($P < 0.05$) in mean scores for degenerative or necrotic lesions, fibrosis, and lipidosis as the degree of white striping increased from NORM to SEV. The results from the histopathological study were supported by the findings from proximate analysis confirming that the fat and protein contents of muscle increased ($P < 0.05$) and decreased ($P < 0.05$), respectively, as the degree of white striping increased. In conclusion, the histopathological changes occurring in white striping indicate a degenerative myopathy that could be associated with increased growth rate in birds.

Key words: white striping, pathology, proximate analysis, myopathy, broiler breast fillet

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IMMUNOLOGY, HEALTH, AND DISEASE

Pathological changes associated with white striping in broiler breast muscles

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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. This study was undertaken to evaluate the details of changes in histology as well as proximate composition occurring in the fillets with respect to the 3 degrees of white striping. In experiment 1, representative breast fillets for each degree of white striping (n = 20) were collected from 45-d-old broilers, approximately 2 h postmortem. From each fillet, 2 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. In experiment 2, samples with 3 degrees of white striping were collected from 57-d-old birds for conducting proximate analysis. Major histopathological changes observed in the MOD and SEV samples consisted of loss of cross striations, variability in fiber size, flocular/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 for degeneration and necrosis, fibrosis, and lipidosis. The scale used to score the samples ranged from 0 (normal) to 3 (severe). There was an increase ($P < 0.05$) in mean scores for degenerative or necrotic lesions, fibrosis, and lipidosis as the degree of white striping increased from NORM to SEV. The results from the histopathological study were supported by the findings from proximate analysis confirming that the fat and protein contents of muscle increased ($P < 0.05$) and decreased ($P < 0.05$), respectively, as the degree of white striping increased. In conclusion, the histopathological changes occurring in white striping indicate a degenerative myopathy that could be associated with increased growth rate in birds.

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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 cases of white striping evaluated by consumers (Kuttappan et al., 2012c). 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; Klasing, 2008). 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). The details of tissue changes taking place in birds with white striping need to be explored to determine whether it is a variation within a normal physiological range or a muscle abnormality. According to Valentine and McGavin (2012), the cytoarchitectural characteristics of skeletal muscle are best evaluated by microscopic examination. We hypothesize that there may be differences in microscopic lesions between the NORM, MOD, and SEV degrees of white striping that could have resulted in a change in proximate composition reported by Kuttappan et al. (2012a). 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, a 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 derive speculations about the etiology of the tissue changes associated with the occurrence of white striping.

**MATERIALS AND METHODS**

**Experiment 1**

Muscle samples for the histologic study were collected from birds processed at 45 d 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 10 h before 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. Because 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., 2012c). 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 NORM. Those fillets with white striations generally <1 mm thick, but readily observed...
on the fillet surface were considered to have a MOD degree of white striping. The SEV fillets had thick white striations (generally >1 mm thick) that 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. 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 4 µm, 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 2). The microscopic lesions included floccular/fibrillar and 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 that 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. Both acute and chronic (polyphasic) changes were observed in same tissue sections. 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 is a statistical increase in the severity of the lesions.

**Statistical Analysis**

The myopathic lesions, fibrosis, and lipidosis scores as well as the proximate composition (dorsal or ventral portions) of the muscle samples were analyzed to evaluate the differences between NORM, MOD, and SEV degrees of white striping. The data were analyzed using ANOVA (SAS Institute Inc., Cary, NC), and means were separated with Tukey’s honestly significant difference test with P < 0.05 considered as significant. Individual birds were considered as the experimental unit for the entire analysis.

**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 toward the cranial end where the fillet was thicker compared with the caudal portion (Figure 1). 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, whereas 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 (Figure 2). 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 that 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. Both acute and chronic (polyphasic) changes were observed in same tissue sections. 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.
was a significant ($P < 0.05$) increase in the occurrence of chronic myopathic lesions along with lipidosis and fibrosis (Figure 3). Furthermore, the histopathological score from different muscle samples showed that the fillets (dorsal and ventral surfaces) 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 (from NORM to MOD and SEV) of the breast fillets (Table 1). This histopathological observation was in accordance with the gross lesions observed in the respective muscle samples as well.

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 4). 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 2 had a higher ($P < 0.05$) fat content compared with NORM. Furthermore, the ventral surfaces of the MOD and SEV fillets had a higher ($P < 0.05$) fat content than the corresponding dorsal surfaces, whereas there was no difference in fat content

Figure 2. 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 (hematoxylin and eosin staining; a, b), fibrosis (Masson’s trichrome; c, d), and lipidosis (Oil red O; e, f). Panel b: arrow indicates a representative region showing degeneration of muscle fibers along with infiltration of inflammatory cells. Panel d: arrow indicates fibrous tissue (stained with blue color in the online PDF) infiltrating the interstitial space. Panel f: arrow indicates fat tissue (dark area or red color in the online PDF). Color version available in the online PDF.
due to location within the NORM fillets. In addition, as the fat content increased associated with an 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 compared with that of the MOD and SEV fillets (Figure 5). 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 6. 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.

**DISCUSSION**

The present study indicates that the severe degrees of white striping are histopathologically characterized by chronic myopathic lesions. The polyphasic (both acute and chronic) changes in the same sections suggested continuous and ongoing exposure to the causative insult (Valentine and McGavin, 2012). According to Dubowitz (1985), myopathic lesions are nonspecific, which could be occurring due to several 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. Previous studies on white striping suggest that it could be an emerging condition that is closely associated with the increased growth rate and heavier breast fillets in broilers (Bauermeister et al., 2009; Kuttappan et al., 2012a,b, 2013). Wilson et al. (1990) reported

**Figure 3.** Histopathological scores for myopathic lesions (hematoxylin and eosin staining), fibrosis, and lipidosis in fillets from different degrees of white striping \((n = 20\) for each degree of white striping). \(^a-c\)Means within each histopathological characteristic with different letters are significantly different \((P < 0.05)\). NORM = normal (no white striping); MOD = moderate degree of white striping; SEV = severe degree of white striping.

**Figure 4.** Fat content in breast fillets with different degrees of white striping \((n = 15/each degree of white striping)\). \(^a-d\)Means with different letters are significantly different \((P < 0.05)\). NORM = normal (no white striping); MOD = moderate degree of white striping; SEV = severe degree of white striping.
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 deep pectoral myopathy or inherited muscular dystrophy. There are reports of muscular damage in turkeys that 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). Furthermore, the lack of sufficient blood supply could result in accumulation of metabolic waste products (MacRae et al., 2006), leading to oxidative stress and tissue damages. The increased levels of serum enzymes indicating muscle damage along with myopathic changes was observed in birds with higher BW (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 several 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

Figure 5. Protein content in breast fillets with different degrees of white striping. a–cMeans with different letters are significantly different (P < 0.05). NORM = normal (no white striping); MOD = moderate degree of white striping; SEV = severe degree of white striping.

Figure 6. Correlation between the mean (average of dorsal and ventral portions of the fillet) fat and protein in breast fillets with NORM (black diamonds), MOD (open squares), and SEV (crosses) degrees of white striping. NORM = normal (no white striping); MOD = moderate degree of white striping; SEV = severe degree of white striping.
release of factors such as cytokines, prostaglandins, and so on (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., 2000). 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 overstretching or ischemia in tissues, resulting in muscle damage and initiation of reparative responses. Later, these reparative or regenerate attempts failed and took a different phase leading to fatty degeneration. This could be why the MOD and SEV fillets have a higher lipidosis and fibrosis compared with the NORM. The presence of the acute and chronic myopathic lesions in the same tissue confirms the chronic ongoing insult that may have resulted in ineffective regenerative process. It should be noted that the gross lesions are more pronounced toward 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 compared with 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 differences 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), whereas 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 toward 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 consumers (Kuttappan et al., 2012c). So, further studies, along with comparison of white striping to various known muscular abnormalities in poultry, 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 a decrease in protein and an increase in fat percentages. The occurrence of tissue changes is more frequent toward the craniao-ventral surface of the fillet compared with the other regions. Furthermore, lesions are more apparent in pectoralis major (fillets) and iliotibialis muscle compared with the pectoralis minor (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.

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