Poultry Science

Broiler breast muscle myopathies: association with satellite cells

Sandra G. Velleman [1]

Department of Animal Sciences, The Ohio State University, Wooster, OH 44691, USA

ABSTRACT Heavy weight fast-growing meat-type broiler chickens have largely been selected for growth rate, muscle mass yield especially for the breast muscle, and feed conversion. Substantial improvements have been made, but in recent years breast meat quality issues resulting in product downgrades or condemnation have occurred especially from necrotic and fibrotic myopathies like Wooden Breast. In general, the morphological structure of the broiler breast muscle has changed in the modern commercial broiler with muscle fiber diameters increased, circulatory supply decreased, and connective spacing between individual fibers and fiber bundles decreased. Satellite cells are the primary cell type responsible for all posthatch muscle growth, and the repair and regeneration of muscle fibers. Recent evidence is suggestive of changes in the broiler satellite cell populations which will limit the ability of the satellite cells to regenerate damaged muscle fibers back to their original. These changes in the cellular biology of broiler satellite cells are likely associated with the necrosis and fibrosis observed in myopathies like Wooden Breast.

Key words: breast muscle, broiler, chicken, pectoralis major muscle, satellite cell, Wooden Breast

2023 Poultry Science 102:102917
https://doi.org/10.1016/j.psj.2023.102917

INTRODUCTION

Meat quality results from the morphological structure and cellular biology regulating the growth and development of skeletal muscle. For the commercial poultry industry, the breast muscle (pectoralis major muscle; **p. major**) is economically the most valuable muscle. Body weight gain and breast muscle yield have been 2 key considerations in genetic selection strategies. In recent years, heavy weight fast-growing broilers have been reported to have incidence of the following myopathies: White Striping; Spaghetti Meat; and Wooden Breast.

White Striping is characterized by white striations of fat and connective, the strips are in parallel to the breast muscle myofibers. White Striping is associated with increased growth rate of broilers with almost all heavy weight commercial birds in the United States having some degree of White Striping (Kuttappan et al., 2012). Although White Striping does not affect the quality of the broiler breast meat product, skinless broiler breast filets have reduced consumer acceptance due to the white striations. In contrast to the white striations that characterize White Striping, Spaghetti Meat results from a loss of breast muscle integrity due to the connective tissue not maintaining muscle structural integrity (Baldi et al., 2018; Soglia et al., 2021). The term spaghetti refers to the myofibrillar rod structure which can be separated from the intact muscle. The etiology of Spaghetti Meat is still not well understood.

The remainder of this article will focus on the fibrotic and necrotic myopathy Wooden Breast in broilers and the potential role of the adult myoblast stem cell, satellite cell, in the propagation of this myopathy. The Wooden Breast myopathy was originally described by Sihvo et al. in 2014. Birds affected with Wooden Breast are phenotypically characterized with muscle discoloration, a turbid viscous coating of the outer muscle surface, and a palpably hard texture (Sihvo et al., 2014). At the microscopic level, the p. major muscle has significant muscle fiber necrosis, small regenerating muscle fibers, and extensive fibrosis with collagen deposition and fat accumulation (Velleman and Clark, 2015). The reports associating heritability with this myopathy have been variable (Bailey et al., 2015; Alnahhas et al., 2016). The exact cause of Wooden Breast remains unclear, but both hypoxia and oxidative stress (Abasht et al., 2016; Soglia et al., 2019) and an etiology like type 2 diabetes in mammals (Lake et al., 2019; Lake and Abasht, 2020) have been hypothesized as possible causes.

At the cellular level, Wooden Breast has been detected as early as 2 wk of age (Chen et al., 2019). Thus, early cellular development of the breast muscle is important in the onset of the Wooden Breast myopathy. Immediately after hatch the only cell responsible for

Received June 23, 2023.
Accepted June 28, 2023.

[1]Corresponding author: velleman.1@osu.edu

muscle growth is the satellite cell. Satellite cells were first reported by Mauro (1961) and are closely positioned adjacent to the sarcolemma of the muscle fiber. The growth of skeletal muscle occurs through the embryonic formation of muscle fibers by hyperplasia of the myoblasts. At the time of hatch, myofiber formation is complete (Smith, 1963) and subsequent muscle growth is mediated by the hypertrophic enlargement of existing myofibers through a precisely regulated process of hypertrophy. As well as the satellite cells mediating hypertrophic growth, satellite cells are responsible for the repair and regeneration of muscle in response to injury. The repair of existing myofibers should result in the formation of new myofibers identical to the original muscle fiber.

Function of Satellite Cells in the Regeneration of Myofibers

Satellite cell-mediated repair results in the regeneration of existing myofibers or the generation of new myofibers. The regeneration process is initiated when the sarcolemma (plasma membrane) of the myofiber is damaged and creatine kinase levels increase. With the degeneration of the myofiber, there is influx of calcium into the myofiber with the activation of calpain. Once the myofiber undergoes necrosis, the cellular debris is phagocytized with an influx of neutrophils and macrophages.

For satellite cell repair mechanisms to regenerate the damaged myofiber, the satellite cells must first be activated and return to the cell cycle from a state of quiescence. After the growth period, satellite cells, in general, will withdraw from the cell cycle and remain in a state of quiescence. Upon activation, the cells must then be able to proliferate and differentiate into muscle specific structures. The environment surrounding the satellite cells, satellite cell niche, will affect satellite cell activity including proliferation and differentiation through its composition of extracellular matrix proteins, growth factors, and vascular supply (Christov et al., 2007; Bi and Kuang, 2012). Satellite cells need to be within 21 μm of capillaries to proliferate and sufficient vascularization is needed for muscle to regenerate (Christov et al., 2007; Rhoads et al., 2009).

The p. major muscle is a fast twitch anaerobic muscle and has lower vascularization due to it being an anaerobic muscle compared to oxidative slow-twitch muscles (Velleman, 2015). As selection has increased breast muscle mass largely based on hypertrophic growth resulting in larger diameter muscle fibers, the connective tissue spacing needed to separate fiber bundles and for circulatory supply has been starved out. When myofibers and fiber bundles begin to touch one another, degeneration begins (Velleman et al., 2003). If the amount of circulatory supply is insufficient, satellite cell mediated repair will be impeded affecting the regeneration of myofibers back to their original state.

Another factor affecting satellite cell-mediated regeneration of muscle is their heterogeneity. Satellite cells are not a uniform population of cells but are a dynamic stem cell population with variable proliferation and differentiation rates (Kuang et al., 2007). Changes in the satellite cell population over time can include the expression of cell surface receptors (Xu et al., 2023) which will modify the interaction of the cell with its satellite cell niche environment. Although the heterogeneity of satellite cells is not completely understood, we do know that satellite cells are not one homogenous population even within a same fiber type muscle like the p. major muscle (McFarland et al., 1995).

Effect of Selection for Breast Muscle Mass Accretion on Satellite Cell Activity in Broilers

Wooden Breast affected breast muscle is characterized by myofiber necrosis, inflammatory cell infiltration, and fibrosis (Sihvo et al., 2014; Velleman and Clark, 2015). Breast meat quality is the direct result of the morphological structure and cell biology of the muscle. Faster growing heavy weight broilers have a higher degree of degenerating muscle fibers associated with the presence of excessively enlarged myofibers from satellite cell-mediated hypertrophic growth. In commercial poultry, selection for growth has largely been determined post-hatch when growth is from the satellite cells not the myoblasts. Satellite cell growth is through the donation of their nuclei to existing fibers increasing protein synthesis capabilities of existing fibers resulting in fiber enlargement not the generation of new fibers. For heavy weight fast-growing broilers, the increase in breast muscle mass has been correlated with larger muscle fiber cross sectional area due to hypertrophic growth (Berri et al., 2007).

As cross-sectional area of the myofibers has increased both circulatory supply and available connective tissue spacing have been significantly reduced. In the case of Wooden Breast affected birds, oxidative stress has greatly increased (Abasht et al., 2016). With the increase in myofiber diameter, when fibers and fiber bundles begin to make contact, degeneration occurs evoking satellite cell-mediated repair mechanisms. To regenerate damaged and necrotic myofibers, the satellite cells will be activated to proliferate and differentiate forming a new fiber identical to the originating one. The regeneration process is well regulated. To reassemble a muscle fiber requires the formation of parallel array of microfibrils which are composed of repeating units of contractile sarcomeres. The primary components of the sarcomere are the Z disks, actin thin filaments and myosin thick filaments. The regeneration of the muscle fiber is completely dependent upon the activity of satellite cells. Thus, if satellite cell proliferation and differentiation is suppressed, muscle fiber regeneration will be negatively impacted affecting breast meat quality.

In the case of Wooden Breast, Velleman et al. (2018) showed in affected p. major muscle that the diameter of the myofibrils was significantly smaller with disorganization of sarcomeric structure. Figure 1 contains

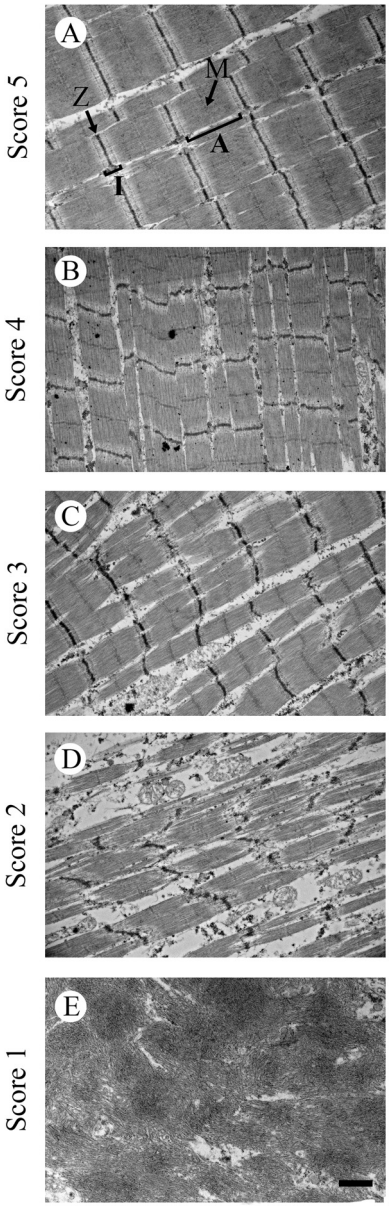

Figure 1. Representative transmission electron microscopy images for sarcomere organization scoring from 1 to 5. (A) Score of 5 shows sarcomere with normal structure; (B) score of 4 means mildly altered; (C) score of 3 means further disorganization from a score of 4 but still moderate; (D) score of 2 means severely altered sarcomere structure; and (E) score of 1 indicates complete absence of sarcomere structure. A = A zone; M = M line; Z = Z line. Bar = 1 μm. Reproduced from Velleman et al. (2018).

representative images illustrating the scoring of sarcomere structure as it relates to the organization of the sarcomere. The high degree of smaller diameter fibers with sarcomere disorganization is of significant concern regarding the ability of Wooden Breast affected muscle to regenerate muscle fibers back to their original state. Thus, in Wooden Breast, degenerating and necrotic muscle will lead to fibrosis with the replacement of muscle with extracellular matrix proteins including collagen and proteoglycans, and fat deposition. The structural stability of muscle including its ability to stretch and hold water is determined by the extracellular matrix composition. Fibrosis results in an overproduction of primarily the fibrillar collagens, Types I and III, but it is the organization especially the crosslinking of these collagens once they form collagen fibers that will determine the effect on meat tenderness.

To further understand the impact of collagen organization on the broiler breast meat, a series of research studies were undertaken by the Velleman group. Collagen fiber structure was accessed in 3 fast-growing commercial broiler lines with varying detection of Wooden Breast by palpation. The lines are identified as Lines A, B, and C. Detection of Wooden Breast by palpation was high in Line A, intermediate in Line B, and not detectable in Line C. In Line A with Wooden Breast detectable by palpation, the collagen fibers were closely packed in parallel arrays whereas Line B had a diffuse structure of the collagen fibers (Figure 2; Velleman and Clark, 2015; Velleman et al., 2017). Interestingly, microscopic examination of the p. major muscle showed that 70% of the birds in the study had necrosis and fibrosis with only a small percentage scored as being Wooden Breast positive by hand palpation. Thus, hand palpation and scoring will only detect those birds with a stiffened and hard breast muscle, but not with microscopically detectable necrosis and fibrosis.

Effect of Selection for Growth on Satellite Cell Activity in Broilers

Growth selection strategies have been similar in commercial chickens and turkeys (Havenstein et al., 2003, 2004, 2007), but p. major muscle necrotic and fibrotic myopathies like Wooden Breast have not been reported to date in heavy weight fast-growing meat-type turkeys (Zampiga et al., 2020). Since the ability to regenerate muscle fiber structure is dependent on satellite cell activity and suppressed in current commercial broilers, the question arises as to how selection for growth has affected satellite cell function in heavy weight commercial chickens compared to the turkey. Thus, myopathies like Wooden Breast may be associated with differences in the myogenic and regenerative potential of p. major muscle satellite cells. As shown by Xu et al. (2021), satellite cells isolated from the p. major muscle of modern commercial turkeys have greater proliferation and differentiation compared to satellite cells isolated from historic slower growing turkeys representing older commercial lines.

To study how selection for growth has been potentially altered satellite cells isolated from a modern commercial broiler line was compared to a Cornish Rock and Randombred chicken lines from the 1990s (Xu and Velleman, 2023). Modern commercial broiler satellite cells had decreased proliferation and differentiation compared to the Cornish Rock and Randombred chicken lines. In contrast, modern heavy weight fast-growing p. major muscle cells from a meat-type turkey have significantly higher proliferation and differentiation compared to the historic slower growing commercial line turkey satellite cells (Xu et al., 2021). These data are strongly

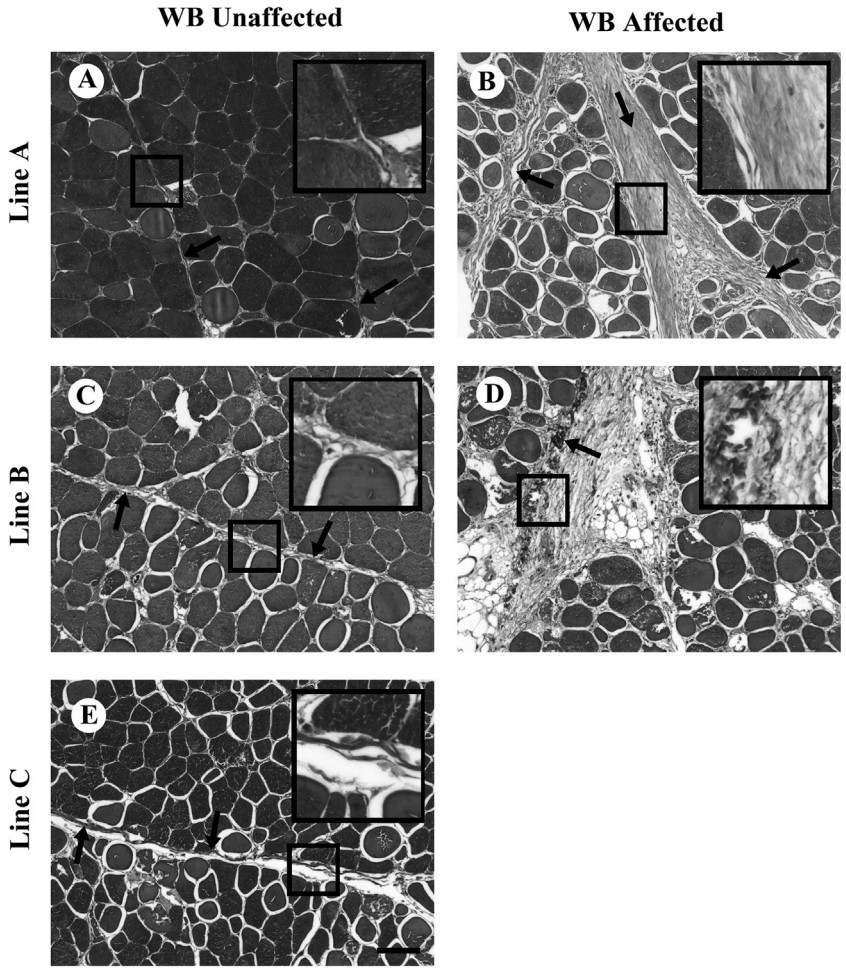

Figure 2. Masson trichrome staining of collagen organization in Wooden Breast-unaffected and -affected pectoralis major muscle. (A, C, and E) are representative images of Wooden Breast-unaffected pectoralis major muscle from Lines A, B, and C, respectively. (B, D) are representative images of Wooden Breast-affected pectoralis major muscle from Lines A and B, respectively. The arrows highlight fibrillar collagen and the boxes contain enlargements of the fibrillar collagen. Scale bar = 100 μm. Reproduced from Velleman et al. (2017).

suggestive that modern commercial meat-type chickens have decreased myogenic activity of their satellite cells which would reflect in decreased regenerative potential that may be associated with the development of necrotic and fibrotic myopathies like Wooden Breast. As put forth by Bailey (2023), industry management strategies are now placing emphasis on maintaining optimal levels of satellite cell proliferation immediately after hatch to aid in the control of p. major muscle myopathies.

Effect of Selection for Breast Muscle Yield: Fast Compared to Slower Growing Broilers

In recent years, there has been a consumer move toward heritage type or slower growing meat-type broilers as their meat quality are perceived to be better. This perception is, in part, driven by meat quality issues like Wooden Breast, the phenotypic appearance being negatively impacted by White Stripping, and the large size of the p. major muscle. Despite conditions like Wooden Breast having a low heritability (Bailey et al., 2015), how the p. major muscle grows during hyperplasia and hypertrophic growth must be monitored as these growth periods will determine the morphological structure of the muscle. It is certainly attractive to believe that moving toward slower growing birds will address many of the breast muscle pathophysiological conditions affecting meat quality in broilers. These conditions like Wooden Breast are recent in occurrence and associated with heavy weight fast-growing broilers. Since meat quality is the result of the morphological structure of the muscle and the cellular growth of the muscle cells, selection practices need to begin to include more muscle cell biology. Inclusion of muscle cellular biology will likely reduce the incidence of detrimental myopathies negatively impacting meat quality. Thus, approaches utilized in selection programs should encompass both muscle growth from hyperplasia and hypertrophy, and the overall muscle fiber organization of the breast muscle.

The activation, proliferation, and differentiation of satellite cells is a critical area that must be evaluated in the selection of birds to reduce incidence of degenerative, necrotic and fibrotic myopathies. As shown by Xu and Velleman (2023), the proliferation and differentiation potential of satellite cells has significantly declined in modern commercial broilers compared to older lines from the 1990s. Wooden Breast, for example, is

characterized by persistent damage to the muscle fibers and inflammation, instead of the fibers being able to regenerate back to their original state they are replaced with fat and connective tissue including highly cross-linked fibrillar collagen. The impact of reduced satellite cell activity is supported by Wooden Breast-affected muscle having a greater amount of small myofibers (Clark and Velleman, 2017) with disorganization of sarcomere structure (Velleman et al., 2018).

CONCLUSIONS

As the broiler industry moves forward, necrotic and fibrotic breast muscle myopathies like Wooden Breast will continue to remain an area of focus. Despite the desire to identify one cause or remedy, strategies utilized will need to be multifaceted. As discussed here, understanding of the cell biology of muscle growth and regeneration is critical in approaches to reduce the incidence of these myopathies and to gain knowledge of their etiology.

ACKNOWLEDGMENTS

This symposium contribution did not receive any grant funding from agencies in the public, commercial, or not-for-profit sectors.

DISCLOSURES

No conflict of interest is declared.

REFERENCES

Abasht, B., M. F. Mutyrn, R. D. Michalke, and W. Lee. 2016. Oxidative stress and metabolic perturbations in wooden breast disorder in chickens. PLoS One 11:e0153750.

Alnahhas, N., C. Berri, M. Chabault, P. Chartrin, M. Boulay, M. Bourin, and E. Le Bihan-Duval. 2016. Genetic parameters of white striping in relation to body weight, carcass composition, and meat quality traits in two broiler lines divergently selected for the ultimate pH of the pectoralis major muscle. BMC Genetics 17:61.

Bailey, R. A. 2023. Strategies and opportunities to control breast myopathies: an opinion paper. Front. Physiol. 14:1173564.

Bailey, R. A., K. A. Watson, S. F. Bilgili, and S. Avendano. 2015. The genetic basis of pectoralis major myopathies in modern broiler chicken lines. Poult. Sci. 94:2870–2879.

Baldi, G., F. Soglia, M. Mazzoni, F. Sirri, L. Canonico, E. Babini, L. Laghi, C. Cavani, and M. Petracci. 2018. Implications of white striping and spaghetti meat abnormalities on meat quality and histological features in broilers. Animal 12:164–173.

Berri, C., E. Duval-Le Bihan, M. Debut, V. Santé-Lhoutellier, E. Baéza, V. Gigaud, Y. Jégo, and M. J. Duclos. 2007. Consequence of muscle hypertrophy on characteristics of pectoralis major muscle and breast meat quality of broiler chickens. J. Anim. Sci. 85:2005–2011.

Bi, P., and S. Kuang. 2012. Stem cell niche and postnatal muscle growth. J. Anim. Sci. 90:924–935.

Chen, L. R., M. M. Suyemotoa, A. H. Sarsourb, H. A. Cordovab, E. O. Oviedo-Rondón, M. Wineland, H. J. Barnesaand, and L. B. Borst. 2019. Temporal characterization of wooden breast myopathy ("woody breast") severity and correlation with growth rate and lymphocytic phlebitis in three commercial broiler strains and a random-bred broiler strain. Avian Pathol. 48:319–328.

Christov, C., F. Chrétien, R. Abiu-Khalil, G. Bassez, G. Vallet, F. J. Authier, Y. Bassaglia, V. Shinin, S. Tajbakhash, B. Chazaud, and R. K. Gherardi. 2007. Muscle satellite cells and endothelia cells: close neighbors and privileged partner. Mol. Biol. Cell 18:1397–1409.

Clark, D. L., and S. G. Velleman. 2017. Spatial influence on breast muscle morphological structure, myofiber size, and gene expression associated with the wooden breast myopathy in broilers. Poult. Sci. 95:2930–2945.

Havenstein, G., P. Ferket, J. Grimes, M. Qureshi, and K. Nestor. 2004. Performance of 1966 vs. 2003-type turkeys when fed representative 1966 and 2003 turkey diets. Proc. World's Poult. Congr..

Havenstein, G., P. Ferket, J. Grimes, M. Qureshi, and K. Nestor. 2007. Comparison of the performance of 1966-versus 2003-type turkeys when fed representative 1966 and 2003 turkey diets: growth rate, livability, and feed conversion. Poult. Sci. 86:232–240.

Havenstein, G., P. Ferket, and M. Qureshi. 2003. Carcass composition and yield of 1957 versus 2001 broilers when fed representative 1957 and 2001 broiler diets. Poult. Sci. 82:1509–1518.

Kuang, S., K. Kuroda, F. Le Grand, and M. A. Rudnicki. 2007. Asymmetric self-renewal and commitment of satellite stem cells in muscle. Cell 129:999–1010.

Kuttappan, V., V. Brewer, J. Apple, P. Waldroup, and C. Owens. 2012. Influence of growth rate on the occurrence of white striping in broiler breast fillets. Poult. Sci. 91:2677–2685.

Lake, J. A., and B. Abasht. 2020. Glucolipotoxicity: a proposed etiology for wooden breast and related myopathies in commercial broiler chickens. Front. Physiol. 11:169.

Lake, J. A., M. B. Papah, and B. Abasht. 2019. Increased expression of lipid metabolism genes in early stages of wooden breast links myopathy of broilers to metabolic syndrome in humans. Genes 10:746.

Mauro, A. 1961. Satellite cell of skeletal muscle fibers. J. Biophys. Biochem. Cytol. 9:493–495.

McFarland, D. C., K. K. Gilkerson, J. E. Pesall, J. S. Walker, and Y. Yun. 1995. Heterogeneity in growth characteristics of satellite cell populations. Cytobios 82:21–27.

Rhoads, R. P., R. M. Johnson, C. R. Rathbone, X. Liu, C. Temm-Gove, S. M. Sheehan, J. B. Hoying, and R. E. Allen. 2009. Satellite cell-mediated angiogenesis in vitro coincides with a functional hypoxia-inducible factor pathway. Am. J. Physiol. Cell Physiol. 296:C1321–C1328.

Sihvo, H. K., K. Immonen, and E. Puolanne. 2014. Myodegeneration with fibrosis and regeneration in the pectoralis major muscle broilers. Vet. Pathol. 51:619–623.

Smith, J. H. 1963. Relation to body size and number in the chicken. Poult. Sci. 42:619–623.

Soglia, F., M. Mazzoni, and M. Petracci. 2019. Spotlight on avian pathology: current growth-related breast meat abnormalities in broilers. Avian Pathol. 48:1–3.

Soglia, F., M. Petracci, R. Davoli, and M. Zappaterra. 2021. A critical review of the mechanisms involved in the occurrence of growth-related abnormalities affecting broiler chicken breast muscles. Poult. Sci. 100:101180.

Velleman, S. G. 2015. Relationship of skeletal muscle development and growth to breast muscle myopathies: a review. Avian Dis. 59:525–531.

Velleman, S. G., J. W. Anderson, C. S. Coy, and K. E. Nestor. 2003. Effect of selection for growth rate on muscle damage during turkey breast muscle development. Poult. Sci. 82:1069–1074.

Velleman, S. G., and D. L. Clark. 2015. Histopathologic and myogenic gene expression changes associated with wooden breast in broiler breast muscles. Avian Dis. 59:410–418.

Velleman, S. G., D. L. Clark, and J. R. Tonniges. 2017. Fibrillar collagen organization associated with the broiler wooden breast fibrotic myopathy. Avian Dis. 61:481–490.

Velleman, S. G., D. L. Clark, and J. R. Tonniges. 2018. The effect of the wooden breast myopathy on sarcomere structure and organization. Avian Dis. 62:28–35.

Xu, J., G. M. Strasburg, K. M. Reed, N. M. Bello, and S. G. Velleman. 2023. Differential effects of temperature and mTOR and Wnt-planar cell polarity pathways on syndecan-4 and CD44 expression in growth-selected turkey satellite cell populations. PLoS One 18:e0281350.

Xu, J., G. M. Strasburg, K. M. Reed, and S. G. Velleman. 2021. Response of turkey pectoralis major muscle satellite cells to hot

and cold thermal stress: effect of growth selection on satellite cell proliferation and differentiation. Comp. Biochem. Physiol. Pt. A. 252:110823.

Xu, J., and S. G. Velleman. 2023. Effects of thermal stress and mechanistic target of rapamycin and wingless-type mouse mammary tumor virus integration site family pathways on the proliferation and differentiation of satellite cells derived from the breast muscle of different chicken lines. Poult. Sci. 102:102608.

Zampiga, M., F. Soglia, G. Baldi, M. Petracci, G. M. Strasburg, and F. Sirri. 2020. Muscle abnormalities and meat quality consequences in modern turkey hybrids. Front. Physiol. 11: 554.