Mitochondrial characteristics of chicken breast muscle affected by wooden breast

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Objectives: The novel myopathy called "wooden breast" affects meat quality in commercial broiler chickens. Histological findings have also reported degeneration of muscle fibers, muscle damage and infiltration of phagocyte into the muscle degeneration site. Wooden breast occurs with excessive oxidative stress; reactive oxygen species, which cause oxidative stress, are mainly produced in mitochondria. Mitochondria are essential organelles; they account for 90% of cellular oxygen consumption and contribute to most ATP synthesis. The mitochondrial electron transport chain is involved in tissue oxygen consumption and generates reactive oxygen species. Mitochondria contain two lipid bilayers that constitute the outer and inner membranes, and this structure is vulner- able to oxidative damage by reactive oxygen species because it is rich in phospholipids and proteins. Reactive oxygen species generated in the body are enzymatically removed to maintain homeostasis. However, in skeletal muscle, it can be difficult to maintain homeostasis because of an abnormality that occurs in the reactive oxygen species removal system, resulting in excessive oxidative stress. Breast muscle affected by wooden breast has substantially higher reactive oxygen species levels and lower cytochrome C oxidase activity localized in the outer mitochondrial membrane compared with unaffected breast muscle; thus, affected breast mus- cle may be abnormal mitochondrial function. In this study, we hypothesized that wooden breast is triggered by mitochondrial dys- function. Therefore, we conducted a study in which wooden breast muscle was classified by severity, and the ultrastructure of mitochondria and their functions were assessed to determine the relationship between mitochondrial function and the seriousness of wooden breast.

Materials and Methods: A total of 36 Ross 308 chickens were raised to 50 days of age. The sampling site was chosen as the most hardened and fibrotic ventral cranial position. For the histological investigation, a cryostat was used to prepare a frozen section 10 μm thick, stained with hematoxylin and eosin (HE) and nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), and then examined for muscle tissue with an optical microscope. The classification of wooden breast severity was based on the circu- larization of muscle fibers reported as a histological feature of the condition in a previous study. Muscle fiber circularity was mea- sured using ImageJ from the HE-stained image of each sample. For mitochondrial observation, a confocal laser scanning micro- scope was used. Primary antibodies against the mitochondrial membrane ATP synthase subunit beta, which is a mitochondrial marker, and laminin antibodies against the basement membrane were used. For TEM observation, the tissue block was sliced into 100-nm-thick sections perpendicular to the long axis of the myofiber using an ultramicrotome. The area of the mitochondria was calculated using Image J. Antioxidant enzyme gene expression were used quantitative real-time polymerase chain reaction. Data were normalized against the expression level of actin beta and analyzed using the ΔCt method for correlation analysis and the ΔΔCt method for stage-related comparison of gene expression.

Results and Discussion: The severity of wooden breast was classified into the three groups based on the circularization of muscle fibers. There was a significant difference in the circularity of muscle fibers among these groups. NADH-TR staining revealed an in- crease in darkly stained muscle fibers as wooden breast severity. Immunohistochemistry indicated that red particles, indicating the presence of mitochondria were observed on the margins of muscle fibers in normal samples. In contrast, mitochondria were observed throughout the muscle fibers in wooden breast-mild and -severe samples. In addition, red particles were larger in severe cases compared with those in normal and mild cases. Ultrastructure observation of normal breast muscle revealed a small number of mitochondria with a distinct cristae structure. Conversely, wooden breast-mild and -severe breast muscle did not have mitochondria with a clear cristae structure. Furthermore, mitochondria swelled with the increasing severity of wooden breast; the respective mitochondrial areas for normal, mild, and severe cases were 0.098 ± 0.055, 0.155 ± 0.143, and 0.249 ± 0.183 μm2, with a significant difference detected among all groups ($p < 0.001$). The severity of wooden breast was compared against the expression levels of various antioxidant enzyme genes. The expression of superoxide dismutase 1 (SOD1) differed significantly among wooden breast severity levels, with the highest gene expression level observed in normal tissue. The expression of superoxide dismutase 2 (SOD2) differed significantly between normal and wooden breast-severe cases, with normal tissue again showing higher gene expression.

Conclusions: Breast muscle severely affected by wooden breast showed decreased mRNA levels of the genes that encode the SOD1 and SOD2 antioxidant enzymes. In wooden breast muscle, the levels of reactive oxygen species, which should be maintained at a constant level, are increased, thereby causing damage in various membrane tissues of the cell and structural disruption of mem- brane organelles such as mitochondria.

Key words: Wooden breast, Broiler, Antioxidant enzyme