PRESENCE AND DISTRIBUTION OF PROCOLLAGEN AND COLLAGEN TYPE III IN CHICKEN *PECTORALIS MAJOR* AFFECTED BY MUSCULAR ABNORMALITIES

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I. INTRODUCTION

The selection programs carried out in the past 50 years aiming at developing high growth-rate and breast-yield chicken hybrids resulted in an overall improved production profitability [1] and led, from the other side, to an increased incidence of abnormalities mainly affecting the *Pectoralis major* (PM) muscles [2]. Among them, having a remarkably higher incidence and exerting detrimental effects on quality traits and technological properties of meat, the White Striping, Wooden Breast and Spaghetti Meat defects are of relevant importance. As recently reviewed [2], the microscopic observations carried out to evaluate the histological features associated with the occurrence of these muscular abnormalities evidenced profound degenerative myopathic changes involving the replacement of necrotic fibers with an extensive proliferation of connective tissue (fibrosis). In this context, being typically observed in developing/regenerating muscles [3], collagen type III and its immature form (procollagen type III) might likely be involved in the occurrence of White Striping, Wooden Breast and Spaghetti Meat abnormalities. Thus, the present study aimed at evaluating the presence and distribution of procollagen and collagen type III in PM muscles from broiler hybrid and an unselected breed. In addition, to elucidate whether these features are altered by the occurrence of the current growth-related breast abnormalities, PM affected by White Striping, Wooden Breast and Spaghetti Meat abnormalities were considered.

II. MATERIALS AND METHODS

Twelve *Pectoralis major* (PM) samples (3 muscles/group) were selected from the same flock of fast-growing broilers (males belonging to Ross 308 strain, slaughtered at 45 day-old and having an average live weight of 3.0 kg) classified by macroscopical lesion as Normal (NORM), White Striping (WS), Wooden Breast (WB) and Spaghetti Meat (SM). In addition, two PM samples were collected from an egg-laying breed (cocks belonging to Leghorn, slaughtered at 160 day-old and having an average live weight of 2.5 kg) and considered as a control group (C). Samples for immunohistochemical analyses were excised from the superficial section of the cranial portion of the PM muscle and quickly frozen in isopentane. Serial cross-sections (10 µm-thick) were cut on a cryostat microtome at -20°C and mounted on poly-L-lysine coated glass slides. For immunohistochemistry, the Avidin-Biotin-peroxidase Complex (ABC) method and 3,3'-diaminobenzidine chromogen were used to visualize the immune reaction against procollagen type III and collagen type III antibodies.

III. RESULTS AND DISCUSSION

The immunohistochemical observations performed on PM belonging to C group showed normal endomysial and perimysial connective tissues organized as a very thin layer and thicker collagenous *septa*, respectively (Fig. 1 A, B). Immunoreactivity to procollagen type III was almost absent and only fibroblasts were intensely stained (Fig. 1 A, arrow). Concurrently, collagen type III was intensely marked and appeared normally arranged within the endomysial and perimysial compartments (Fig. 1 B, arrow). On the other hand, a profound modification of the connective tissue architecture, involving a different presence and distribution of procollagen and collagen type III, was observed in PM from the fast-growing genotype affected by muscular

abnormalities (Fig. 1 A2, B2, A3, B3, A4, B4) and even within those muscles exhibiting a macroscopically normal appearance (Fig. 1 A1, B1). In addition, degenerated muscle fibers were replaced by diffused and poorly organized connective tissue immunoreactive to both procollagen and collagen type III (Fig. 1 A2 and B2, arrowheads). With regard to NORM, immunoreactivity to procollagen was restricted to some areas of the perimysial spaces (Fig. 1 A1, arrow), collagen type III resulted intensely labelled in the endomysium, whereas in the perimysial septa intensely stained areas were intermingled to weakly marked ones (Fig. 1 B1, arrow). The immunoreactivity for procollagen and collagen type III observed for the endomysial and perimysial connective tissue in WS muscles suggested an increased collagen synthesis taking place (Fig. 1 A2, B2). The weak positivity of both procollagen and collagen type III observed in WB (Fig. 1 A3, B3, arrows), in which intensely stained endomysial spaces were intermingled to faintly labeled perimysial compartments, was in agreement with the immunohistochemical features previously observed in hypertrophic muscles. In detail, this result might be explained by the changes in amino acid composition formerly observed in collagen isolated from dystrophic chickens, in which a selective removal of the polar side-chains and their replacement with non-polar amino acids was found [4]. As for SM (Fig. 1 A4, B4), the progressive rarefaction of the endomysial and perimysial connective tissue resulted in a changed procollagen type III immunoreactivity and suggested an altered collagen turnover and synthesis. Indeed, immunoreaction to procollagen was almost absent while the endomysial connective tissue exhibited a strong collagen III labelling thus demonstrating an alteration of the collagen composing the perimysial compartment that might account for the distinctive macroscopic features associated to this abnormality in which a detachment of the muscle fiber bundles composing the PM is normally found.

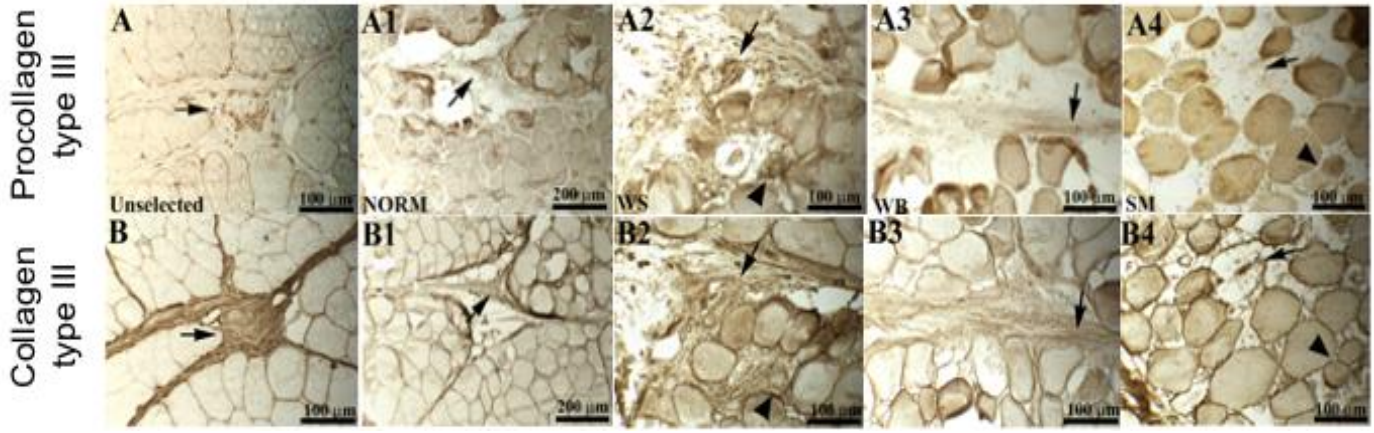

Figure 1. Serial cross-sections showing the immunoreaction to procollagen type III and collagen type III antibodies of chicken *Pectoralis major* muscles belonging to the control group (A, B) as well as broiler hybrid exhibiting macroscopically normal appearance (A1, B1) and affected by WS (A2, B2), WB (A3, B3) and SM (A4, B4) abnormalities.

IV. CONCLUSION

The profound alteration in the presence and distribution of both procollagen and collagen type III observed in fast-growing broiler hybrid likely results in impaired quality traits, nutritional value and technological properties of meat that shift from the distinctive features normally found in chicken meat.

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