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Pectoral Vessel Density and Early Ultrastructural Changes in Broiler Chicken Wooden Breast Myopathy

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Summary

In wooden breast myopathy (WBM) of broiler chickens, the pectoralis major muscles show abnormally hard consistency and microscopical myodegeneration of unknown aetiology. To date, previous studies have focused primarily on chronic WBM and ultrastructural descriptions of early WBM are lacking. The aim of this study was to elucidate the pathogenesis of WBM by light microscopical morphometry of vessel density and the ultrastructural description of early WBM changes with transmission electron microscopy. The pectoral vessel density was compared between unaffected chickens ($n = 14$) and two areas of focal WBM in affected chickens ($n = 14$). The transverse myofibre area per vessel was highest in the unaffected area of muscle from cases of focal WBM, significantly higher ($P = 0.01$) than in macroscopically unaffected tissue, indicating that relatively decreased blood supply may trigger the development of WBM. The ultrastructural study included unaffected chickens ($n = 3$), two areas of focal WBM from affected chickens ($n = 3$) and areas of diffuse WBM from affected chickens ($n = 3$). The morphologically least affected myofibres within the WBM lesion areas in light microscopy exhibited ultrastructural changes of increased sarcoplasmic reticulum diameter and mitochondrial hyperplasia. Such changes originate typically from osmotic imbalance, for which the most likely aetiologies in WBM include tissue hypoxia or myodegeneration of the surrounding myofibres. The findings suggest that a relative reduction of blood supply in the major pectoral muscle occurs in the early phase of WBM, which may be linked to the ultrastructural changes of osmotic imbalance.

Keywords: broiler chicken; electron microscopy; vessel density; wooden breast myopathy

Introduction

Wooden breast myopathy (WBM) of broiler chickens manifests as a hardened consistency and pale colour of the major pectoral muscle and is characterized microscopically as polyphasic myodegeneration, accompanied by fibrosis in the chronic phase (Sihvo *et al.*, 2014). WBM starts to develop as a focal lesion at approximately 2 weeks of age (Sihvo *et al.*, 2017), but the aetiology and pathogenesis are currently unknown. In slaughter-age broilers of approximately 5–6 weeks of age, the WBM lesion has typically progressed into a chronic and diffuse phase, completely affecting the major pectoral muscle.

Recent hypotheses for WBM aetiopathogenesis include tissue hypoxia and oxidative stress; the genes associated with these conditions show increased expression in chronic cases of WBM (Mutryn *et al.*, 2015; Abasht *et al.*, 2016). Other studies of chronic WBM suggest differences in connective tissue composition as an aetiology or point to a multifactorial aetiology including altered glucose metabolism, unbalanced calcium ion homeostasis, disturbed muscle fibre development and others (Velleman and Clark, 2015; Zambonelli *et al.*,

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2016). However, chronic WBM involves prominent tissue repair processes and secondary changes, such as fibrosis (Sihvo *et al.*, 2014). Accordingly, an analysis of the early changes in WBM in younger birds would be of value in elucidating the pathogenesis of WBM. Recently, the role of lymphocytic phlebitis in WBM has been speculated (Papah *et al.*, 2017; Sihvo *et al.*, 2017).

The major pectoral muscle of broiler chickens consists almost entirely of fast glycolytic type IIb fibres (Remignon *et al.*, 1995; Papinaho *et al.*, 1996; MacRae *et al.*, 2006). In rapidly growing high-yield broilers, the type IIb fibres show increased diameter (hypertrophy) compared with slower-growing broilers or layer chickens (Remignon *et al.*, 1995; Soike and Bergmann, 1998a; MacRae *et al.*, 2006; Velleman and Clark, 2015; Clark and Velleman, 2016). Large muscle fibre diameter is speculated to link the high occurrence of muscle pathologies in high-yielding broilers to metabolic stress in the muscle. This is due to an increased diffusion distance for oxygen and other metabolites from the blood vessels to the centre of the myofibre (Soike and Bergmann, 1998a).

In addition to the higher initial myofibre diameter compared with layers, the myofibre diameter increases with age in high-yielding broilers, while the total vessel density decreases (Joiner *et al.*, 2014; Radaelli *et al.*, 2017). Capillaries, rather than other vessels, appear to increase in number in response to the myofibre hypertrophy in order to maintain the necessary blood supply to growing muscle (Joiner *et al.*, 2014; Radaelli *et al.*, 2017). Insufficient vessel number, either initially or due to the failure to increase the vessel number following myofibre hypertrophy, could further promote oxidative stress in the muscle. A definite relationship between microvessel number and WBM, however, remains to be established.

Currently, both the acute (Papah *et al.*, 2017; Sihvo *et al.*, 2017) and chronic lesions of WBM (Sihvo *et al.*, 2014, 2017; de Brot *et al.*, 2016; Soglia *et al.*, 2016) have been described at the level of light microscopy, but ultrastructural descriptions of WBM and broiler skeletal muscle in general are sparse (Soike and Bergmann, 1998b; Papah *et al.*, 2017). Ultrastructural changes associated with degeneration of skeletal muscle in general are well known. The first alterations include reduced myofibrillar width with normal sarcomere structure retained, while more progressed degeneration is characterized by Z-line alterations, destruction of sarcomeres and increased amounts of sarcoplasm that replace the sarcomeres (Ghadially, 1997a). These ultrastructural degenerative changes do not directly suggest any specific aetiopathogenesis, but electron microscopy is a valuable tool to exclude certain muscle diseases and to gather indirect evidence of metabolic or oxidative stress.

The aims of the present study were to evaluate microvessel density in the major pectoral muscle in unaffected chickens and chickens with focal WBM by light microscopy and to describe the ultrastructure of early WBM by transmission electron microscopy by comparing the unaffected chickens with birds having focal and diffuse WBM.

Materials and Methods

Animals and Macroscopic Evaluation

The birds of this study originated from two flocks (A and B) of high-yielding and fast-growing, commercial hybrid broiler chickens. The birds, 240 male broiler chickens in flock A and 350 in flock B, were transferred from a commercial hatchery to an experimental rearing facility of the University of Helsinki within their first day of life. The unvaccinated 1-day-old birds were randomly divided in woodchip-bedded pens, 10 (flock A) or 14 birds (flock B) per pen and provided with water and feed *ad libitum*. The base of the feed consisted of wheat and soya bean and 0.007% narasin (Monteban, Elanco, Greenfield, Illinois, USA), which was included as a coccidiostat. The lighting programme included 6 h of darkness daily, except for the first 7 days when heating lamps were used. The broilers were weighed, killed by mechanical cervical dislocation and exsanguinated via cervical blood vessels before necropsy examination and macroscopical evaluation for WBM as previously described (Sihvo *et al.*, 2017). Briefly, the major pectoral muscles were graded as unaffected (normal muscle consistency), focal WBM (muscle consistency hardened in a demarcated area, surrounded by areas of normal consistency) or diffuse WBM (the complete muscle area hardened). Since the birds were not subject to ante-mortem experimental procedures, legislation applicable to conventional breeding was followed in rearing and killing of the birds. The methods of rearing and killing were approved by the Laboratory Animal Centre of the University of Helsinki.

Microvessel Density

The microvessel density study included 28 birds from flock A. The stratified random selection of the 28 broilers (14 unaffected and 14 with focal WBM) was at the ages of 18 days ($n = 3$), 24 days ($n = 5$), 35 days ($n = 4$) and 38 days ($n = 2$). The right major pectoral muscle was sampled in the middle area (unaffected chickens) or in the lesion area and in the unaffected area (chickens with focal WBM),