Wooden breast lesions in broiler chickens in the United Kingdom S. de Brot ¹, S. Perez², H.L. Shivaprasad³, K. Baiker¹, L. Polledo¹, M. Clark⁴, L. Grau-Roma ¹ ¹School of Veterinary Medicine and Science (SVMS), University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom (UK) ² Minster Veterinary Practice, Salisbury Road, York YO26 4YN ³ California Animal Health and Food Safety Laboratory System, Tulare branch, University of California-Davis, CA 93274, USA ⁴ Minster Veterinary Practice, College Road, Sutton Bonington, Leicestershire LE12 5RA Address correspondence to Dr. Llorenç Grau Roma (llgrau@nottingham.ac.uk)

Abstract

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A new condition causing significant economic losses due to lesions in the *pectoralis major* muscle of commercial broiler chickens has been recently reported in Finland. The condition has been named wooden breast and is characterized by its gross and histological appearance. Between October 2014 and April 2015, samples from 5 pectoralis major muscles downgraded in the slaughterhouse were submitted to the Veterinary Pathology Service of the University of Nottingham for histological examination. All the five studied samples showed moderate or severe multifocal polyphasic muscular degeneration and necrosis. All cases showed variable degree of interstitial fibrosis and/or presence of adipose tissue within interstitium, as well as formation of small nodular follicle-like aggregates of lymphocytes adjacent to small blood vessels. These lesions are compatible to wooden breast and overlap with another recently reported condition known as white striping. The present communication provides evidence that wooden breast lesions are present in broiler chickens in the UK and discusses its similarities with white striping. Larger studies assessing the prevalence of wooden breast are needed to determine the relative economic importance of this condition in this country.

42 A new condition affecting the *pectoralis major* muscle of commercial broiler chickens named 43 wooden breast has been recently reported in Finland (Shivo and others 2014). This condition is reported to cause significant economic losses because it causes rejection from human 44 45 consumption (Shivo and others 2014, Trocino and others 2015). Wooden breast is characterized by its gross and histological appearance. Grossly, pectoral muscles are hard, out 46 bulging and pale. Polyphasic muscular degeneration with variable degrees of interstitial 47 48 fibrosis and presence of perivenular lymphocytic aggregates are the histological features. Since its first description, several reports have suggested a wide occurrence of this condition 49 50 in Europe and the United States of America (USA) (Mudalal and others 2015, Mutryn and others 2015, Shivo and others 2014). However, as far as the authors are aware, there are no 51 peer-reviewed reports of wooden breast in the United Kingdom (UK). 52 Between October 2014 and April 2015, pectoralis major muscle samples from 5 broiler 53 54 chickens (cases No. 1 to 5) coming from 3 different farms were submitted to the Veterinary Pathology Service of the University of Nottingham for histological examination. The 55 56 submitted muscles corresponded to downgrades in the slaughterhouse. No other skeletal muscles from chickens belonging to those slaughter batches were downgraded. All chickens 57 were 49-50-days-old Ross 308 broilers chickens of commercial flocks with typical in-farm 58 mortality rates and without any out-standing clinical problems. The muscles were 59 downgraded by meat inspectors and reported to be pale and hard. Samples were received 60 fixed in 10% formalin. Grossly, pathologists noted that the tissues appeared diffusely pale 61 and, in two of the five cases (cases No. 2 and 3), evident thin white striations were observed 62 63 between the muscular fibres (Figure 1). Three to 4 longitudinal and transverse sections were trimmed of each sample and routinely processed for histological examination. From each 64 sample, two 5 µm-thick sections were cut and stained with Haematoxylin and eosin (H&E) 65 stain and Masson's trichrome stain. Histological lesions were assessed by two ECVP board 66

certified pathologists (SdB, LGR). Several parameters were semiquantified on the most affected tissue section and given semi-quantitative scores as follows: i) muscular degeneration and necrosis: 0 = absence; 1 = less than 10% of fibres affected (mild); 2 = between 10 and 40% of fibres affected (moderate); 3 = more than 40% fibres affected (severe); ii) interstitial fibrosis: 0 = absence; 1 = mild and multifocal; 2 = moderate and multifocal; 3 = abundant and diffuse or multifocal; iii) presence of adipose tissue within the interstitium: graded from low to abundant, similarly as for interstitial fibrosis. In addition, the presence or absence of the following features was noted: myotube formation, lymphocytic aggregates adjacent or around small blood vessels (with or without vessel wall being affected) and presence of inflammatory cells within and around necrotic fibres. The degree of fibrosis was semi-quantified by using Masson's trichrome stain; while rest of the lesions were assessed in H&E stained slides. All the five studied samples showed moderate or severe multifocal polyphasic muscular degeneration and necrosis, characterized by hyalinization, swelling, loss of cross striations, fragmentation and/or incipient mineralization of skeletal muscle fibres. Macrophages and heterophils were often infiltrating and/or surrounding the necrotic fibres. Myotube formation, a feature indicating regeneration, was also observed in all the cases studied although it was minimal in case No. 3. The degree of interstitial fibrosis was scored as moderate in 3 cases (cases No. 1, 4 and 5), mild in one (case No. 2) and absent in another one (case No. 3). Case No. 3, instead, showed the presence of moderate amounts of proteinaceous material within the interstitium (interpreted as oedema). Variable amounts of adipose tissue were present in all cases, being abundant in case No. 2 and 3, moderate in cases No. 1 and 5, and low in case No. 4. In all the cases, the adipose tissue was present around the blood vessels as well as in other areas of the interstitium, apparently replacing muscle fibres. Finally, the formation of small nodular follicle-like aggregates of lymphocytes adjacent to or surrounding small blood

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vessels (mainly venules) was present in all the cases, with the lymphocytes occasionally invading the blood vessel walls (Figure 2).

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Altogether, the gross and histological appearance of the studied cases is compatible to wooden breast (Shivo and others 2014). However, this condition is reported to have overlapping features with another also recently described muscular condition, initially in the USA and later in Europe, affecting broiler chickens, known as white striping (Kuttappan and others 2012). The latter is described to affect mainly pectoral muscles and thighs. Both conditions show polyphasic muscular degeneration and necrosis as a common feature, but the presence of lymphocytic aggregates, observed in all the 5 here presented cases, is suggested to be exclusive of the condition wooden breast (Shivo and others 2014). Although the cause of both conditions remains unknown, several studies have suggested that their pathogeneses is associated to an increased body weight and growth rate (Kuttappan and others 2012, Russo and others 2015, Shivo and others 2014, Velleman and others 2015), and evidences of localized hypoxia have been provided by RNA-sequencing analysis of affected muscles (Mutryn and others 2015). Considering the strong gross and histopathological similarities and suggested pathogenesis, it seems likely that both conditions correspond to different manifestations of the same entity (Petracci and others 2015, Russo and others 2015). Actually, cases No. 2 and 3 also showed evident intramuscular white striations, the typical gross feature of white striping (Kuttappan and others 2012). According to Shivo and others (2014), only the lymphocytic aggregates next to the venules would differentiate them from the previously described white striping (Shivo and others 2014). Nevertheless, a recent study from Italy reported the presence of lymphofollicular infiltrates within the interstitium of breasts affected by white striping (Russo and others 2015). In the authors' opinion, if future studies demonstrate that both entities definitely correspond to the same disease, a unique and

general term as for example "chicken breast myopathy" might be more adequate to refer to both conditions. The present communication provides evidence that wooden breast lesions are present in broiler chickens in the UK. Larger studies assessing the prevalence of this condition and the number of downgraded or rejected pectoral muscles in the UK slaughterhouses are needed to determine the relative economic importance of this condition in this country. Acknowledgements The authors thank Alan Lasslett and Jo Sanders for their excellent technical assistance. The research leading to these results has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme (FP7/2007-2013) under REA grant agreement No PCOFUND-GA-2012-600181.

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Figure legends:

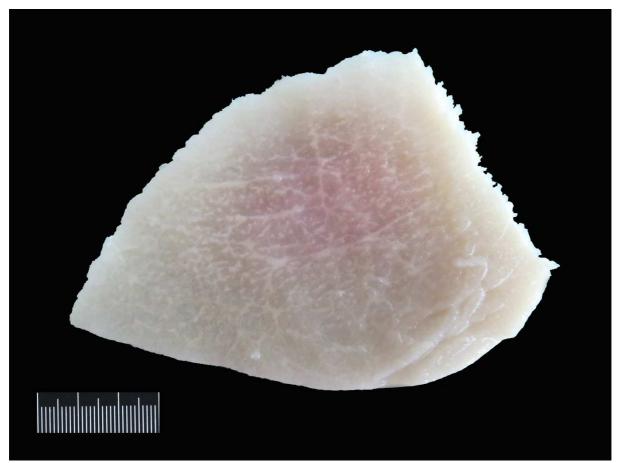


Figure 1. Case No. 3. Transverse section of a formalin-fixed pectoral muscle. Evident multifocal white striations are present between muscular fibres. The ruler indicates millimetres (mm).

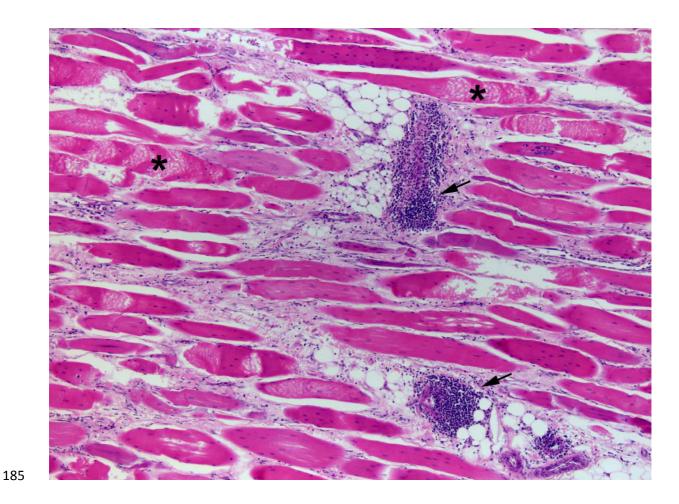


Figure 2. Microphotograph of the major pectoral muscle. Case No. 4. Aggregates of lymphocytes are present adjacent to and infiltrating small vessels (arrows). The muscle fibres often show features of degeneration and necrosis (asterisks). Haematoxylin and eosin (H&E) stain. 10x.