New vascular structure in broiler breast: breast-vessel

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ABSTRACT Chickens intensively selected for accelerating growth rate and enhanced muscle mass has resulted in the occurrence of breast alterations with a negative consumers perception. Together with the already known breast abnormalities (White Striping, Wooden Breast and Spaghetti Meat) a new one has been described by the consumers as a "long and thin worm". For this reason, the aim of this work was to macroscopically and microscopically describe this structure in broiler breast. To this end, 2 different experiments were performed. In the first one, 4 broiler breasts from a supermarket were analysed. Thereafter, a second experiment was carried out on an experimental farm simulating field management conditions in order to confirm the results of the first experiment. To this end, 120 chickens were reared in an experimental farm up to 42 d. Breast samples were collected and analysed macroscopically and microscopically to assess the abnormality on the broiler breast. In both experiments, the results of the analysis showed the finding of tubular structures located on the surface of *pectoralis major* muscle compatible with arteries. So far, currently, this occurrence has not been described in the literature and we propose calling this new broiler breast feature as breast-vessel.

Key words: broiler, breast, vascular structure, pectoralis major

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INTRODUCTION

Close to 123 million tonnes of poultry meat were produced in 2018 over the world and it is likely to increase in the following years (FAO, 2019). The lasts decades have witnessed intensified poultry production in response to the growing global population demand for affordable and high-quality animal protein (FAO, 2019). As a consequence, 135 million tons of chicken meat are expected to be produced in 2025 worldwide (FAO, 2020). In Europe, Spain is the third producer of poultry meat, producing close to 1 million tons (FAO, 2019). To meet growing consumer demand, over the past years, the broiler industry has intensely selected a number of meat-type chicken strains, with the result that today's broiler have, accelerating growth rate and enhanced muscle mass potential (Bodle et al., 2018; Malila et al., 2018). This fact has caused the poultry industry to face the occurrence of recent breast alterations concerning consumers negative perceptions during the past decade, such as White Striping (**WS**), Wooden Breast (**WB**), and Spaghetti Meat (**SM**) (Petracci et al., 2019). This myopathies have caused significant economic losses in the poultry sector (Caldas-Cuevas and Owens, 2020; Hafez and Attia, 2020). In spite of the fact of such economical impact, the etiological causes are unknown. The main factor associated to them are the intensive genetic selection for rapid-growth rates and high yields in broiler (Caldas-Cuevas and Owens, 2020). In this context, a new breast negative perception has been described as the presence of a tubular structure visible in the surface of broiler breast *pectoralis major* muscle. This structure is reported by consumers as a "long and thin worm" on the surfaces of the breast once the broiler breasts are processed and packaged, with negative commercial consequences. To the best of our knowledge, this is the first description of this novel tubular structure in the broiler chicken. Thus, this work aimed to macroscopically and microscopically describe this structure in broiler breast.

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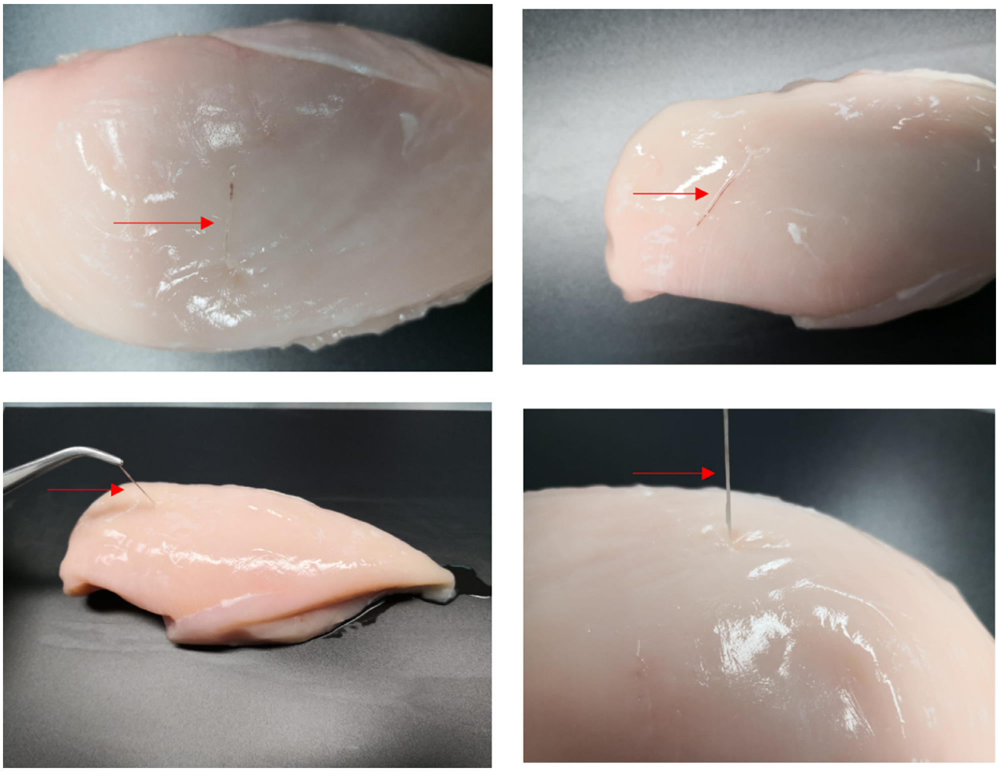

Figure 1. Tubular structure is located on the surface of *pectoralis major* muscle in the broiler breast. The red arrow points to the structure.

Table 1. Weight of the animals (weight ± s.d.) and conversion rate (CR ± s.d.) during the productive cycle.

Week of study	Weight (g)	CR
1	186.32 ± 5.42	1.04 ± 0.00
2	467.13 ± 22.7	1.32 ± 0.02
3	847.93 ± 37.34	1.59 ± 0.03
4	1384.48 ± 62.45	2.01 ± 0.05
5	2055.30 ± 96.15	1.97 ± 0.05
6	2825.37 ± 156.09	2.03 ± 0.05

MATERIALS AND METHODS

Due to consumer claim, in this study, carcasses from a supermarket located in the Valencia Region were analyzed. Hereafter, to rule out randomness, a study was carried out on a experimental farm simulating field conditions. For this purpose, 120 chickens were reared from 1-day-old to 42 d, a whole production cycle. All the animals used in this experiment were handled according to the principles of animal care published by Spanish Royal Decree 53/2013 (Spain, 2013).

Experiment 1. Study Sample From a Supermarket

A total of 4 breasts were removed from the food chain following consumer rejection after visual detection of a tubular structure through shrink wrap plastic from commercial packages. For microscopic study, samples were

Table 2. Composition of the experimental diets.

Nutrient composition (%)	Diet	
Analyzed	Starter	Grower
Crude fat	3.5	3.5
Crude protein	20.5	18.0
Crude fiber	2.6	4.8
Crude ash	6.6	5.5
Lysine	1.1	0.96
Methionine	0.6	0.39
Calcium	1.0	1.0
Phosphorus	0.7	0.4
Sodium	0.2	0.1
Vitamin premix[1]	0.05	0.03
Mineral premix[2]	0.10	0.10
Calculated		
Crude fat	4.2	3.4
Crude protein (Nx6.25)	20.9	17.7
Crude fiber	3.4	5.5
Ash	5.9	5.6
Calcium	1.0	0.8
Phosphorus	0.7	0.4
Ingredients	Corn, soy flour, wheat, soy oil, calcium carbonate, monocalcium phosphates, sodium chloride	Corn, soy flour, rice bran, calcium carbonate, sodium chloride

[1]The following was supplied per kilogram of diet in the starter diet: vitamin A (retinyl acetate), 10,000 IU; vitamin D_3, 2,000 IU; vitamin E (DL-α-tocopheryl acetate), 0 mg; vitamin K 5 mg. The following was supplied per kilogram of diet in the grower diet: vitamin A (retinyl acetate), 5,000 IU; vitamin D_3, 1,000 IU; vitamin E (DL-α-tocopheryl acetate), 30 mg; vitamin K, 3 mg.

[2]The following was supplied per kilogram of diet in the starter diet: Mn, 120 mg; Zn, 100 mg; Fe, 80 mg; Cu, 20 mg; I, 2 mg; Se, 0.3 mg; Co, 0.5 mg. The following was supplied per kilogram of diet in the grower diet: Mn, 100 mg; Zn, 80 mg; Fe, 30 mg; Cu, 15 mg; I, 2 mg; Se, 0.3 mg; Co, 0.2 mg.

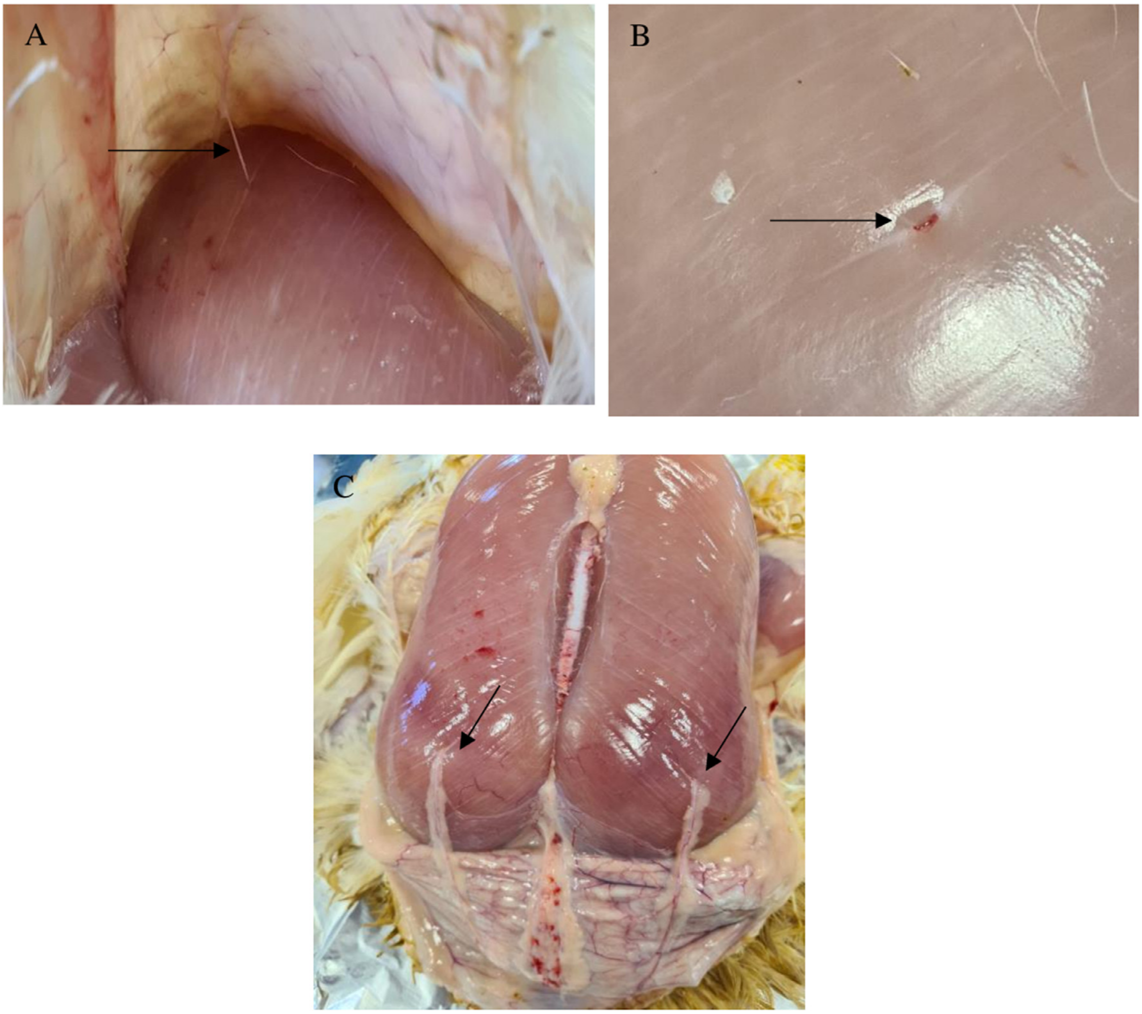

Figure 2. Tubular structure located on the surface of *pectoralis major.* (A) The black arrow points to the structure, skin insertion is observed. (B) The red arrow points to the subtle depression in the third cranial region where the feature starts. (C) The structure surrounded by fat is observed in both breasts.

collected under aseptic conditions by cutting a piece of breast sample where the tubular structure was visible using scalpel and tweezers. Each breast piece was placed into an individual sterile bottle and was fixed by immersion in 10% neutral buffered formalin for minimum 48 h.

At 42 d, animals (n = 120) were slaughter and samples from the breast were taken under sterile conditions. To this end, samples were collected by cutting a piece of breast sample where the tubular structure was visible using scalpel and tweezers. Each breast piece was placed into an individual sterile bottle and was fixed by immersion in 10% neutral buffered formalin for minimum 48 h.

Experiment 2. Study Design in the Experimental Farm

The study was performed in an experimental poultry house in the Center for Animal Research and Technology (CITA, in its Spanish acronym (Valencian Institute for Agrarian Research, IVIA, Segorbe, Spain)). A total of 120-day-old chicks (Ross, Males) provided from the same hatchery were raised to 42 d. Chicks were randomly housed in pens in a final stocking density of 35 kg/m^2, with wood shavings as bedding material. To simulate the real conditions of broiler production, the house was supplied with programmable electric lighting, automated electric heating and forced ventilation. The environmental temperature was set at 32°C on arrival day and gradually reduced to 19°C by 41 d post hatch. The birds received drinking water and feed *ad libitum.* Two different age commercial diets were offered to the animals, a pelleted starter diet from arrival until 21-day-post hatch (Camperbroiler iniciación, Alimentación Animal Nanta, Spain), and pelleted grower diet from 21-day-old to the slaughter day (Alimentación Animal Nanta). Finally, animals were weighed at weekly intervals and feed consumption per pen was recorded.

Microscopic Analysis

A total of 4 and 120 samples from experiment 1 and 2, respectively, were processed equally. To this end, muscle and tubular structure were cutted into ~0.5-cm-thick transverse sections and embedded in paraffin. The samples were sectioned at 2 to 3 mm, processed routinely and stained with hematoxylin and eosin (**H-E**). For this study, selected muscule sections were also stained with Masson's trichrome stain and Weigert Van Gieson stain.

RESULTS

Experiment 1. Study Sample From a Supermarket

Macroscopic Analysis In all the broiler breasts reported by the consumer (n = 4), the tubular structure was located on the surface of *pectoralis major* muscle in the broiler breast, usually starting in a subtle depression in the third cranial region. Red content (blood compatible) occasionally appeared (Figure 1).

Experiment 2. Study Design in the Experimental Farm

No clinical signs were observed during rearing, and the productive parameters obtained were in accordance with the breed standards (Table 1). Nutritional composition of the diets has been detailed in Table 2.

Macroscopic Analysis In all the broiler breasts analysed (n = 120), the tubular structure was located on the surface of *pectoralis major* muscle in the broiler breast, usually starting in a subtle depression in the third cranial region. Red content (blood compatible) occasionally appeared (Figure 2).

Microscopic Analysis Based on the histopathological study, the elongated structures located on the surface of the pectoral muscles corresponded to blood vessels that penetrate the muscle and are covered by a layer of adipose tissue. These blood vessels are composed of tunica intima with a single layer of flattened endothelial cells together with a supporting layer of elastin rich in collagen (internal elastic lamina). Tunica media has abundant amounts of collagen and smooth muscle fibers and a relatively small amount of elastic fibers. The outer layer is the tunica adventitia composed of small amounts of collagen and elastic connective tissue (Figure 3). According to these characteristics, these blood vessels are compatible with muscular arteries. No etiological agent has been observed in the samples analyzed. In addition, all muscle samples analyzed presented different degrees of myodegeneration.

DISCUSSION

Since the 1960s, poultry production has been intensified in response to the growing global population demand for affordable and high-quality animal protein (Chang, 2007). Most of the myopathies have been present prior to 2010 but they have not been recognized in the processing plant, nor recognized as a food safety issue but considered as a quality issue (Bilgili, 2016).

Currently, it is known that the occurrence of WS, WB, and SM is linked to fast-growth rates of the birds and their large breast muscles. The muscle hypertrophy along with an unbalanced growth of supportive connective tissue leads to a compromised blood supply and hypoxia. The occurrence of oxidative stress and mitochondrial dysfunction leads to lipidosis, fibrosis, and

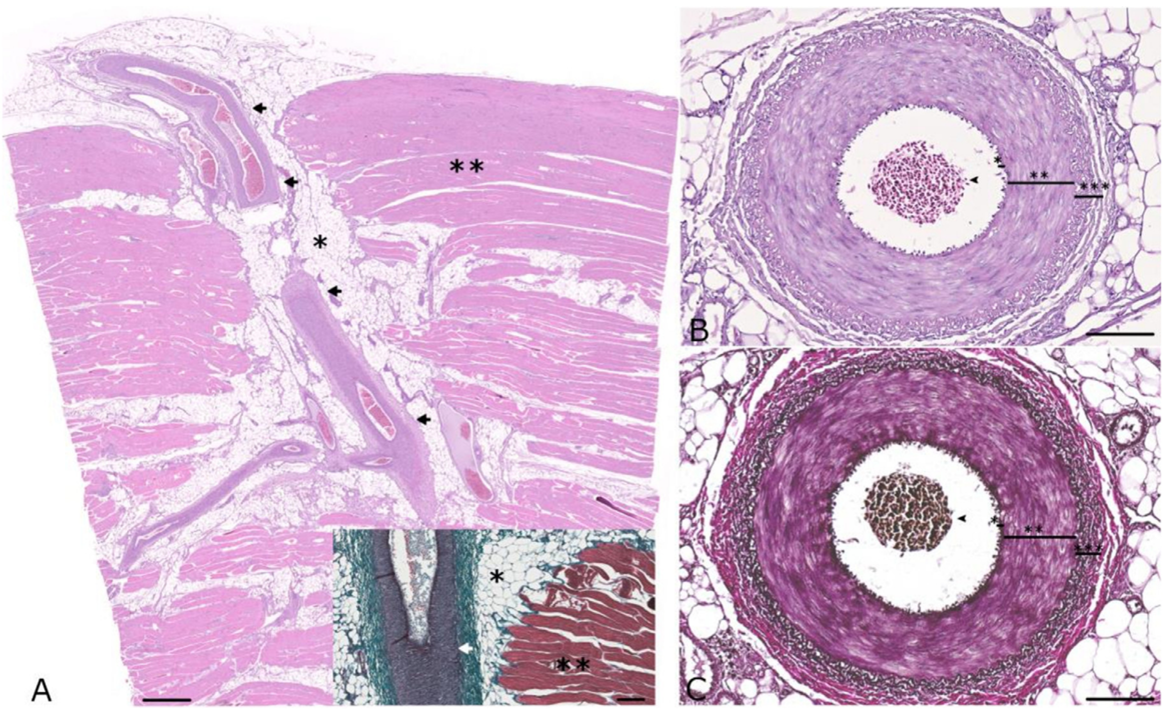

Figure 3. Blood vessel identified on the surface of the breasts. (A) The blood vessel (arrow, longitudinal section) is surrounded by adipose tissue (*) and penetrates the muscle (**). H-E Bar = 500 μm. Inset: detail of the blood vessel (arrow), adipose tissue (*) and muscle fibers (**). Masson's trichrome stain. Bar = 100 μm. (B) Cross section of blood vessel. The tunica intima (*), the tunica media (**) and the tunica adventitia (***) are observed. The lumen of the vessel containing erythrocytes is indicated by arrowhead. H-E Bar = 100 μm. (C) Cross section of blood vessel. Elastic fibers predominate in the tunica intima (*) and the tunica adventitia (***) (black fibers). Muscle fibers predominate in the tunica media (**) (red colored fibers). The lumen of the vessel containing erythrocytes is indicated by arrowhead. Weigert Van Gieson stain. Bar = 100 μm.

overall myodegeneration (Petracci et al., 2019). However, the type of vascular proliferation described in this study has not been related to any of the pectoral pathologies typical of broilers named above, although several degrees of myodegeneration were found.

The pectoral artery originates from subclavian and supplies pectoral musculature. Their major branches are the capillaries of the pectoral muscles. The pectoral veins drain the region of the pectoral musculature and anterior thorax region, and empties to subclavian vein, right and left. Their major sources vessels are capillaries within the pectoral muscles (Proctor and Lynch, 1993).

One hypothesis that could explain the appareance of this undisclosed structure could be as a way to improve the blood supply to this large broiler breast by the modification of the vascular system by vasculogenesis or angiogenesis. Both vasculogenesis, the de novo formation of vessels, and angiogenesis, the growth of new vessels from pre-existing vessels by sprouting, are complex processes that are mediated by the precise coordination of multiple cell types. One of these mechanisms is the hypoxic regulation of angiogenesis (Patel-Hett and D'Amore, 2011). So far, currently, this occurrence has not been described in the literature. This is subject of study as it is critical to identify any myopathy and to define its etiology for a subsequent development of effective strategies to prevent further challenges and improve poultry health, well-being and production sustainability facing poultry industry (Hafez and Attia, 2020). In this regard, and due to the characteristics of the new feature, we propose calling this new broiler breast feature as "breast-vessel".

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DISCLOSURES

No potential conflict of interest was reported by the authors.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2021.101565.

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